厚生労働行政推進調査事業費補助金（新興・再興感染症及び予防接種政策推進研究事業）

ワクチンの有効性・安全性評価と ＶＰＤ (vaccine preventable diseases) 対策への適用に関する 分析疫学研究

平成 26～28 年度　総合研究報告書

平成 29 年 3 月

研究代表者　　廣田　良夫

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区　分	氏　名	所　属	職　名
顧　問	加地　正郎	久留米大学	名誉教授
顧　問	武内　可尚	川崎市立川崎病院	名誉院長
研究代表者	廣田　良夫	医療法人相生会臨床疫学研究センター	センター長
		保健医療経営大学	学長
研究分担者	森　満	札幌医科大学医学部公衆衛生学講座	教授
	星　淑玲	筑波大学医学医療系	研究員
	砂川　富正	国立感染症研究所感染症疫学センター	室長
	浦江　明憲	株式会社メディサイエンスプラニング	代表取締役会長兼社長
	鈴木　幹三	名古屋市立大学　看護学部	客員研究員
	森川佐依子	大阪府立公衆衛生研究所感染症部ウイルス課	主任研究員
	出口　昌昭	市立岸和田市民病院	副院長
	吉田　英樹	大阪市保健所　兼　西成区役所	南部保健医療監/医務主幹
	福島　若葉	大阪市立大学大学院医学研究科公衆衛生学	教授
	大藤さとこ	大阪市立大学大学院医学研究科公衆衛生学	准教授
	中野　貴司	川崎医科大学総合医療センター小児科	教授
	小笹晃太郎	公益財団法人放射線影響研究所 疫学部	部長
	入江　伸	医療法人相生会	理事長
	都留　智巳	医療法人相生会ピーエスクリニック	院長
	岡田　賢司	福岡歯科大学総合医学講座小児科学分野	教授
	井手　三郎	聖マリア学院大学	理事長・学長
	原　めぐみ	佐賀大学医学部社会医学講座予防医学分野	准教授
研究協力者	葛西　健	世界保健機関西太平洋地域事務局（WPRO）	事業統括部長
	山口　真也	霞ヶ浦医療センター小児科	小児周産期診療部長
	大久保一郎	筑波大学医学医療系	教授
	越田　理恵	金沢市保健局	局長
	磯部　充久	さいたま市健康科学研究センター保健科学課代謝免疫係	主任
	松下　雅英	高知大学医学部家庭医療学講座	准教授
	田中好太郎	国立感染症研究所　感染症疫学センター	協力研究員
	前田　章子	大阪市立大学大学院医学研究科公衆衛生学	研究員
	松井　珠乃	国立感染症研究所感染症疫学センター第一室	室長
	菅野　恒治	菅野小児科医院	院長
	高崎　好生	高崎小児科医院	院長
	進藤　静生	医療法人しんどう小児科	理事長
	横山　隆	医療法人横山小児科医院	顧問
	横山　隆人	医療法人横山小児科医院	院長
	山下　祐二	医療法人やました小児科医院	院長
	芝尾　敬吾	医療法人しばおクリニック	院長
	清松　由美	医療法人きよまつ小児科医院	院長
	渡辺　憲治	大阪市立総合医療センター消化器内科	副部長
	山上　博一	大阪市立大学大学院医学研究科消化器内科学	講師
	織田　慶子	保健医療経営大学	教授
	大津　聡子	日本赤十字社和歌山医療センター　感染症科	部長

区　分	氏　名	所　属	職　名
	津村　直幹	久留米大学医学部小児科	講師
	大津　寧	久留米大学医療センター小児科	講師
	田中　征治	久留米大学医療センター小児科	助教
	山崎　和子	埼玉医科大学総合医療センター小児科	医師
	齋藤　智也	国立保健医療科学院健康危機管理研究部	上席主任研究官
	加瀬　哲男	大阪市立大学大学院医学研究科公衆衛生学	研究員
	Timothy M.Uyeki	Centers for Disease Control and Prevention	疫学専門家
	中村　英夫	中村小児科医院	院長
	堤　裕幸	札幌医科大学医学部小児科学講座	教授
	要藤　裕孝	札幌医科大学医学部小児科学講座	准教授
	大西　浩文	札幌医科大学医学部公衆衛生学講座	准教授
	長谷川準子	札幌医科大学医学部公衆衛生学講座	兼任講師
	尚和　里子	札幌医科大学医学部公衆衛生学講座	訪問研究員
	大浦　麻絵	札幌医科大学医学部公衆衛生学講座	助教
	神谷　元	国立感染症研究所感染症疫学センター	主任研究官
	安藤　由香	倉敷市立児島市民病院小児科	医師
	大平　文人	大阪府立精神医療センター	医師
	土橋　酉紀	広島大学大学院医歯薬保健学研究院公衆衛生学	助教
	松本　道明	高知県衛生研究所　保健科学課	技術次長
	八幡裕一郎	国立感染症研究所感染症疫学センター	主任研究官
	齋藤　剛仁	国立感染症研究所感染症疫学センター	研究員
	森畑東洋一	もりはた小児科	院長
	高橋　琢理	国立感染症研究所感染症疫学センター	研究員
	浜田　文彦	医療法人慈孝会 はまだ小児科	院長
	小林　祐介	国立感染症研究所感染症疫学センター	協力研究員
	河上　祥一	医療法人社団 愛育会 福田病院	病院長
	鈴木　浩司	株式会社メディサイエンスプラニング	顧問
	山本　和英	かずクリニック	院長
	菅　栄	医療法人開生会かいせい病院	院長
	中村　敦	名古屋市立大学大学院医学研究科	病院教授
	柴田伸一郎	名古屋市衛生研究所微生物部	部長
	脇本　寛子	名古屋市立大学看護学部	准教授
	太田　千晴	旭労災病院呼吸器科	部長
	加藤　宗博	旭労災病院呼吸器科	主任部長
	宇佐美郁治	旭労災病院呼吸器科	副院長
	今井誠一郎	マックスプランク研究所（ドイツ）留学中	研究生
	足立　暁	笠寺病院呼吸器内科	病棟医長
	岩島　康仁	岩島医院	副院長
	児島　康浩	こじま内科小児科クリニック	院長
	山田　保夫	やまクリニック	院長
	川村　秀和	川村医院	院長
	吉村　邦彦	三井記念病院呼吸器内科	部長

区　分	氏　名	所　属	職　名
	青島　正大	亀田総合病院呼吸器内科	主任部長
	中島　啓	亀田総合病院呼吸器内科	医長
	中森　祥隆	三宿病院　呼吸器科	非常勤医師
	清田　康	三宿病院　呼吸器科	医長
	吉川　理子	三宿病院　呼吸器科	部長
	宮下　修行	川崎医科大学総合内科学１	准教授
	丹羽　俊朗	浜田・浅井医院　呼吸器科	部長
	伊藤　雄二	総合大雄会病院呼吸器内科	部長
	青山　恵美	総合大雄会病院	看護管理師長
	矢野　久子	名古屋市立大学看護学部	教授
	村端真由美	三重大学医学部看護学科	准教授
	川合　淳郎	川合内科・小児科	副院長
	住田千鶴子	稲沢市民病院感染対策室	管理看護師長
	林　嘉光	春日井市民病院安全推進室	ICT委嘱専門医
	伊藤　功朗	京都大学医学部附属病院呼吸器内科	助教
	吉田　敦	東京女子医科大学　感染症科	准教授
	柴田　尚宏	東濃厚生病院呼吸器内科	部長
	小林　裕明	稲沢市民病院循環器内科	医長
	山本　俊信	旭労災病院呼吸器科	医師
	加藤　研	大同病院呼吸器内科	部長
	大見　純代	大阪市立十三市民病院医療安全対策室	感染管理認定看護師
	宮川　浩一	みやがわクリニック	理事長
	白石　訓	大阪市立十三市民病院呼吸器内科	部長
	藤岡　雅司	ふじおか小児科	院長
	松下　享	松下こどもクリニック	院長
	久保田恵巳	くぼたこどもクリニック	院長
	武知　哲久	武知小児科内科	院長
	八木　由奈	八木小児科	副院長
	松永　一朗	大阪市立大学大学院医学研究科公衆衛生学	特任講師
	近藤　亨子	大阪市立大学大学院医学研究科	技術職員
	廣井　聡	大阪府立公衆衛生研究所感染症部ウイルス課	主任研究員
	中田　恵子	大阪府立公衆衛生研究所感染症部ウイルス課	主任研究員
	古山　将康	大阪市立大学大学院医学研究科女性生涯医学	教授
	橘　大介	大阪市立大学大学院医学研究科女性生涯医学	准教授
	松本　佳也	市立岸和田市民病院産婦人科	主任医長
	西村　貞子	市立岸和田市民病院産婦人科	主任医長
	三原　由佳	宮崎県立宮崎病院小児科	小児科医師
	本村知華子	国立病院機構福岡病院小児科	小児科医長
	西村　直子	江南厚生病院こども医療センター	副院長
	藤野　元子	済生会中央病院　小児科	副医長
	吉川　哲史	藤田保健衛生大学小児科	教授
	宮田　章子	さいわいこどもクリニック	院長

区　分	氏　名	所　属	職　名
	堤　千代	聖マリア学院大学看護学部	教　授
	中村　和代	聖マリア学院大学看護学部	教　授
	古賀　正久	聖マリア病院中央臨床検査センター	室　長
	中尾　友美	聖マリア学院大学看護学部	准教授
	井手悠一郎	聖マリア学院大学看護学部	講　師
	布井　清秀	聖マリア病院	糖尿病センター長
	佐藤　雄一	聖マリア病院・糖尿病内科	診療部長
	坂西　雄太	佐賀大学医学部地域医療支援学講座総合内科部門	講　師
	荒木　薫	佐賀大学医学部社会医学講座予防医学分野	大学院生
	南里　真美	小城市役所　福祉部　健康増進課　母子保健係	母子保健係長/保健師
	麦谷　歩	医療法人相生会墨田病院	医　師
	伊藤　一弥	大阪市立大学大学院医学研究科公衆衛生学	助　教
		医療法人相生会　臨床疫学研究センター墨田病院分室	室　長
	石橋　元規	医療法人相生会ピーエスクリニック	薬剤師
	洲崎みどり	医療法人相生会ピーエスクリニック	看護師
	白源　正成	医療法人相生会博多クリニック	院　長
	井上　恵	医療法人相生会博多クリニック	医　師
	神代　弘子	医療法人相生会博多クリニック	薬剤師
	三浦　由子	医療法人相生会福岡みらい病院臨床研究センター	臨床検査技師
	真部　順子	医療法人相生会福岡みらい病院臨床研究センター	企画部
	平塚　磁郎	医療法人相生会墨田病院	企画部係長
	千色　純子	医療法人相生会墨田病院	看護部主任
	河野　優二	医療法人相生会墨田病院	臨床検査技師
	本間　太一	医療法人相生会墨田病院	臨床検査技師
	森下　宗彦	中日病院呼吸器内科	医　師
	武田　博明	済生会山形済生病院呼吸器内科	部　長
	永坂　博彦	永坂内科医院	院　長
	土田　文宏	済生会山形済生病院呼吸器内科	副部長
	佐藤　千紗	済生会山形済生病院呼吸器内科	副医長
	渡邊　麻莉	済生会山形済生病院呼吸器内科	医　師
	西塚　碧	済生会山形済生病院呼吸器内科	医　師
	坪井　永保	坪井病院	理事長
	村瀬　享子	坪井病院呼吸器内科	医　師
	高橋　由以	坪井病院呼吸器内科	医　師
	中浜　力	中浜医院	院　長
	上田　章人	藤立病院	病院長
	後藤　浩之	ごとう内科クリニック	理事長
	迎　寛	長崎大学病院呼吸器内科	教　授
	宮崎　泰可	長崎大学病院呼吸器内科	講　師
	東山　康仁	北松中央病院	理事長
	塚本　美鈴	北松中央病院呼吸器内科	医　長
共同研究者	小野　優	大阪市立大学大学院医学研究科公衆衛生学	大学院生

区　分	氏　名	所　属	職　名
	松浦　知香	大阪市立大学大学院医学研究科公衆衛生学	大学院生
	三沢　昌史	亀田総合病院呼吸器内科	部長
	大槻　歩	亀田総合病院呼吸器内科	医師
	田中　孝明	川崎医科大学附属川崎病院小児科	講師
	伊東　宏明	亀田総合病院小児科	部長
	田中　敏博	JA 静岡厚生連静岡厚生病院小児科	診療部長
	福島　慎二	東京医科大学病院渡航者医療センター感染制御部・感染症科	助教
	笠井　正志	兵庫県立こども病院 小児感染症科	科長
	山本　裕子	医療法人やまもと医院	副院長
	髙杉　尚志	医療法人髙杉会高杉こどもクリニック	院長
	三宅真砂子	三宅内科小児科医院	副院長
	上田　美子	清音クリニック	副院長
	津川　毅	札幌医科大学小児科	特任講師
	岩切　龍一	佐賀大学医学部内科学講座消化器内科光学医療診療部	診療教授
	白井　慎平	佐賀大学医学部内科学講座消化器内科	助教
	武知茉莉亜	株式会社電通パブリックリレーションズ ヘルスケアコミュニケーションズ1部	
	守田　貴子	公立大学法人福岡女子大学国際文理学部環境科学科	非常勤講師
	野村浩一郎	一般財団法人化学及血清療法研究所　ワクチン事業部門第二製造部	次長
	来海　和彦	一般財団法人化学及血清療法研究所研究部	次長
	松浦　健太	一般財団法人化学及血清療法研究所ワクチン事業部門第二製造部1課	課長
	Emmanuel Vidor	Global Medical Affairs, Sanofi Pasteur SA（France）	Global Medical Expert
	Su Peing Ng	Global Medical Affairs, Sanofi Pasteur SA（France）	Head
	酒井　伸夫	デンカ生研株式会社ワクチン学術部	ワクチン企画推進部長
	丸山　裕一	デンカ生研株式会社ワクチン研究部	部長
	三森　重孝	デンカ生研株式会社ワクチン研究部	部長代理
	五味　康行	一般財団法人阪大微生物病研究会研究開発部門 開発部	部長
	吉井　洋紀	一般財団法人阪大微生物病研究会研究開発部門研究技術部サーベイランス課	課長

厚生労働行政推進調査事業費補助金　（新興・再興感染症及び予防接種政策推進研究事業）
総合研究報告書

ワクチンの有効性・安全性評価と VPD（vaccine preventable diseases）対策への適用に関する分析疫学研究

研究代表者　廣田　良夫　医療法人相生会臨床疫学研究センター長・保健医療経営大学学長

研究要旨

厚労省意向による特定研究

1）定点モニタリング分科会

①　6 歳未満児 821 人（平均 2.7 歳）では、PCR 陽性インフルエンザに対するワクチン接種の調整オッズ比（OR）は、1 回接種で 0.47（95％ CI：0.26-0.85）、2 回接種で 0.49（0.32-0.77）であった（大阪、2013/14 シーズン、症例対照研究 , test-negative design）。

②　6 歳未満児 857 人（平均 2.7 歳）では、PCR 陽性インフルエンザに対するワクチン接種の調整 OR は、1 回接種で 0.59（0.33-1.07）、2 回接種で 0.50（0.31-0.81）であった（大阪、福岡、2014/15 シーズン、症例対照研究 , test-negative design）。

③　6 歳未満児 914 人（平均 2.9 歳）では、PCR 陽性インフルエンザに対するワクチン接種の調整 OR は、1 回接種で 0.67（0.36-1.24）、2 回接種で 0.40（0.26-0.60）であった（大阪、福岡、2015/16 シーズン、症例対照研究 , test-negative design）。

④　6 歳未満児 2,880 人（平均 3.0 歳）では、迅速診断陽性インフルエンザに対するワクチン接種（1 回以上）の調整 OR は 0.72（0.59-0.88）であった。型別にみると、A 型に対する調整 OR は 0.60（0.46-0.78）、B 型に対する調整 OR は 0.78（0.61-0.98）であり、いずれも有意差を認めた（石川、2015/16 シーズン、症例対照研究 , test-negative design）。

2）妊婦健康影響調査分科会

①　妊婦 12,838 人の調査では、10,000 woman-months 当たりの入院率は「非妊娠・流行期」で 1.08「妊娠中・流行期」で 2.54、調整率比（$RR_{M\text{-}H}$）は 4.30（1.96-9.41）であり、妊娠により流行期の入院リスクは 4.30 倍上昇した。特にインフルエンザ関連の基礎疾患を有する者では、妊娠による流行期の入院リスク増加がより顕著（$RR_{M\text{-}H}$ = 6.58）となった（大阪、2010/11 ～ 2013/14 シーズン、self control methods）。

②　妊婦 8,472 人では、インフルエンザ診断に対するワクチン接種の調整 OR は 0.77（0.60-0.98）であった（大阪、2013/14 シーズン、前向き cohort study）。

③　出生児 3,441 人では、インフルエンザ診断に対する「母親のワクチン接種」の調整 OR は、妊娠中の接種で 0.39（0.19-0.84）、出産後の接種で 0.47（0.17-1.28）であった。インフルエンザ入院に対しても「母親のワクチン接種」の調整 OR は 0.27（0.06-1.24）に低下し、境界域の有意差を示した（大阪、2013/14 シーズン、前向き cohort study）。

プロジェクト研究

3）インフルエンザ分科会

【免疫原性】

①　医療法人の職員 156 人（25-65 歳）では、接種前 HI 価＜ 1:40 の者は H1：37％、H3：62％、B：14％であり、うち約半数は接種後に HI 価≧ 1:40 を獲得した（東京、福岡、2014/15 シーズン、前向き cohort study）。

②　2 シーズン連続してワクチン接種を受けた健康成人 141 人（25 ～ 66 歳）では、1 シーズン目に接種後 HI

価 1:40 以上を示した者は、1:40 未満の者に比べて、2 シーズン目の接種後の抗体保有率（sP: sero-protection proportion）が高かった（H1：92％ vs. 28％、H3：94％ vs. 73％、B（山形）：45％ vs. 0％、B（ビクトリア）：53％ vs. 10％）（東京、福岡、2014/15 ～ 2015/16 シーズン、前向き cohort study）。

③　へき地在住高齢者 109 人（平均 76.7 歳、男 39 人）では、2 シーズン連続してインフルエンザワクチンを 2 回接種した場合、特に 76 歳未満の者においては、シーズン終了後まで H3 と B に対する HI 価が維持された（高知、2012/13 ～ 2013/14 シーズン、前向き cohort study）。

④　糖尿病患者 55 人（年齢中央値 65.5 歳）では、インフルエンザワクチンの免疫原性は良好であった（接種後 sP は H1：76％、H3：76％、B：71％）。肥満者（BMI ≧ 25.0kg/m^2）では抗体応答（4 倍以上上昇した者の割合）が良好である一方で、65 歳以上高齢者、HbA1c 高値、AGE 高値の者では抗体応答が低い傾向を認めた（福岡、2014/15 シーズン、前向き cohort study）。

⑤　リウマチ性疾患患者 151 人では、インフルエンザワクチンの免疫原性は良好であった（接種後 sP は H1：84％、H3：73％、B（山形）：73％、B（ビクトリア）：69％）（2015/16 シーズン、前向き cohort study）。

⑥　炎症性腸疾患患者 141 人（平均 42.7 歳、男 80 人）では、インフルエンザワクチン 1 回接種でいずれのワクチン株についても国際基準を満たす良好な抗体応答が得られ（接種後 sP は H1：67％、H3：74％、B（山形）：81％、B（ビクトリア）：83％）、2 回接種による更なる抗体価の上昇は認めなかった（佐賀、2015/16 シーズン、無作為化非盲検対照並行群間比較試験）。

⑦　ネフローゼ患者 49 人（年齢 3 ～ 24 歳、免疫抑制剤使用患者 40 人、RTX 投与患者 9 人）では、RTX 投与患者へのワクチン接種は RTX 投与の 1 ヵ月前に実施することにより RTX の影響を受けずに抗体陽転および抗体上昇が得られる可能性が示唆された（福岡、2014/15 シーズン、前向き cohort study）。

⑧　化学療法中の肺がん患者 25 人では、ワクチン接種後の sP は、H1：84％、H3：84％、B：65％であり、慢性閉塞性肺疾患（COPD）患者 26 人の sP（H1：81％、H3：96％、B：92％）と有意差を認めなかった（千葉、2013/14 シーズン、前向き cohort study）。

【有効性】

⑨　保育園児 629 人では、ワクチン接種（1 回以上）の「インフルエンザ診断」に対する調整ハザード比（HR）は、2011/12 シーズン：0.73（0.53-0.99）、2012/13 シーズン：0.40（0.22-0.71）、2013/14 シーズン：0.74（0.52-1.07）であった（札幌、2011/12 ～ 2013/14 シーズン、後ろ向き cohort study）。

⑩　小学生（4 校：2,223 人）では、ワクチン接種の調整 OR は「A 型インフルエンザ」に対して 0.56（0.42-0.76）であった（土浦市、2014/15 シーズン、前向き cohort study）。

⑪　小学生（4 校：2,278 人）では、ワクチン接種の調整 OR は「A 型インフルエンザ」に対して 0.67（0.45-0.99）、「B 型インフルエンザ」に対して 0.69（0.46-1.02）であった（土浦市、2015/16 シーズン、前向き cohort study）。

⑫　医療法人の職員 155 人（25 ～ 65 歳）では、流行期間中の「迅速診断陽性 A 型インフルエンザ」に対する antibody efficacy は 70％であり、ワクチン有効率は 42％と算出された（東京、福岡、2014/15 シーズン、前向き cohort study）。

4）百日咳分科会

①　DTaP ワクチン接種（1 回以上）の百日咳発症に対する調整 OR は 0.20（0.04-0.97）であった。接種回数別では、1 ～ 3 回接種で 0.15（0.02-1.24）、4 回接種で 0.22（0.04-1.05）であった（2009 ～ 2012 年、症例対照研究）。

②　DTaP ワクチン接種（1 回以上）の百日咳発症に対する調整 OR は 0.06（0.007-0.46）であった。接種回数別では、1 ～ 3 回接種で 0.04（0.003-0.54）、4 回接種で 0.07（0.006-0.78）であった（2012 年 4 月～ 2016 年 11 月、症例対照研究）。

③　15 歳未満児では、DTaP ワクチン接種の百日咳発症に対する粗 OR は、1 ～ 2 回接種で 0.15（0.03-0.70）、3 回接種で 0.13（0.03-0.55）、4 回接種で 0.15（0.04-0.57）であった（高知、2012 年、症例対照研究）。

④　妊婦 792 人では、妊娠中に百日咳含有ワクチンが接種可能なら「接種する」と回答した者は 225 人（28％）

であった（熊本、2016 年、横断研究）。

5）高齢者肺炎分科会（肺炎球菌ワクチン）

① 65 歳以上高齢者では、肺炎に対する調整 OR は肺炎球菌ワクチン 0.76（0.44-1.32）、インフルエンザワクチン 0.79（0.50-1.25）であった。肺炎球菌性肺炎に対する調整 OR は肺炎球菌ワクチン 0.23（0.08-0.66）、インフルエンザワクチン 0.65（0.31-1.36）となり、肺炎球菌ワクチン接種の調整 OR は有意に低下した（2010 年 10 月～ 2014 年 9 月、症例対照研究）。

② 2014 年 10 月、高齢者に対する肺炎球菌ワクチン接種が定期接種化されたことを受けて、肺炎球菌ワクチンの有効性を検討するため、新規に症例対照研究を実施中である（2016 年 10 月～、症例対照研究）。

③ 65 歳以上高齢者では、肺炎に対するコーヒー摂取（1 日 2 杯以上）の予防効果（OR=0.50、95％ CI：0.28-0.88）が示唆された（2009 年 10 月～ 2014 年 9 月、症例対照研究）。

④ 肺炎球菌ワクチンの有効性に関する系統的レビューの結果、PCV13 の有効性を示した論文は 2 編、PPSV23 の有効性を示した論文は 9 編あったが、結果指標によっては関連を認めなかったとする論文も散見された。日本における研究 2 編は、いずれも PPSV23 の有効性を検討した RCT であり、うち 1 編では肺炎や肺炎球菌性肺炎に対して有意なワクチン有効性を示していた。

⑤ 70 代の在宅高齢者 567 人では、肺炎球菌ワクチンが定期接種化される前の 2013、2014 年には肺炎既往や入院・通院歴を有する者がワクチン接種を受けやすく（confounding by indication）、定期接種化（2015 年）された後には介護予防事業参加者やインフルエンザワクチン接種者が肺炎球菌ワクチン接種を受けやすい healthy vaccinee bias が示唆された（北海道、2013 ～ 2015 年、横断研究）。

⑥ 70 歳～ 84 歳の在宅高齢者 546 人では、肺炎球菌ワクチンの定期接種化以降、接種率が上昇傾向にある（2016 年：26％）。接種率の上昇に伴い confounding by indication や healthy vaccinee bias の程度は変化している可能性が示唆された（北海道、2014 ～ 2016 年、横断研究）。

⑦ 70 歳～ 84 歳の在宅高齢者 546 人では、2 年間の追跡調査における死亡者は 21 人、入院・入所者は 18 人であり、肺炎球菌ワクチン接種の「死亡・入院・入所」に対する調整ハザード比（HR）は 0.70（0.21-2.31）であった（北海道、2014 ～ 2016 年、前向き cohort study）。

⑧ 保育園児 632 人では、肺炎球菌ワクチン接種の調整 HR は、急性中耳炎に対して 0.32（0.23-0.44）であった（札幌、2012 年 4 月～ 2014 年 4 月、両向き cohort study）。

6）新規ワクチン検討分科会

① 乳幼児（急性胃腸炎 190 人、うち迅速診断陽性 87 人）では、迅速診断陽性ロタウイルス胃腸炎に対するロタウイルスワクチンの有効率は 72.8％と推計された（佐賀、2012/13 シーズン、case population study）。

② 乳幼児 829 人では、迅速診断陽性ロタウイルス胃腸炎に対するロタウイルスワクチンの調整 OR は、hospital-control との比較で 0.12（0.02-0.91）、test-negative control との比較で 0.13（0.02-1.10）であり、有効率は各々 88％、87％と推計された（佐賀、2014 シーズン、症例対照研究）。

③ 乳幼児 1,067 人では、迅速診断陽性ロタウイルス胃腸炎に対するロタウイルスワクチンの調整 OR は 0.19（0.14-0.27）であり、有効率は 81％（73-86％）と推計された（佐賀、福岡、2015 シーズン、症例対照研究 , test-negative design）。

④ 乳幼児 1,412 人では、迅速診断陽性ロタウイルス胃腸炎に対するロタウイルスワクチンの調整 OR は 0.20（0.14-0.28）であり、有効率は 80％（72-86％）と推計された（佐賀、福岡、2014 ～ 2015 シーズン、症例対照研究 , test-negative design）。

⑤ 米国予防接種諮問委員会（US-ACIP）が 2009 年に刊行したロタウイルスワクチンについての勧告「Prevention of Rotavirus Gastroenteritis Among Infants and Children: Recommendation of the Advisory Committee on Immunization Practice（ACIP）: MMWR 2009; 58: RR-2」を翻訳した。

⑥ 国産の A 型肝炎ワクチン（エイムゲン）を 2 回接種した健康成人 20 人（平均 37.2 歳）では、3 回目接種

として海外製剤の HAVRIX® を接種した場合でも、全例が抗体陽性となり、重篤な副反応は認めなかった（東京、前向き cohort study）。
⑦　国産の A 型肝炎ワクチン（エイムゲン）を 2 回接種した健康成人 12 人（平均 33.7 歳）では、2 回目接種から 3 年以上が経過しても、3 回目接種により全例が抗体陽性となり、重篤な副反応は認めなかった（東京、前向き cohort study）。
⑧　生後 2 ～ 6 ヵ月児 45 人では、市販されている 2 種類の B 型肝炎ワクチン（ビームゲン、ヘプタバックスⅡ）は、いずれの組み合わせにおいても 3 回接種後には全ての対象者が抗体陽性を獲得し、重篤な副反応は認めなかった（福岡、2015 ～ 2016 年、無作為化比較試験）。
⑨　2011 年に実施した「ポリオワクチンの互換性に関する免疫原性試験」の対象児 153 人では、追加免疫後（計 4 回接種後）の抗体保有割合（NA 価≧ 1:8）は 100％に達していた。その後 3 年間の抗体持続を追跡すると、抗体保有割合は、A 群（sOPV → DTaP-sIPV → DTaP-sIPV → DTaP-sIPV）で Wild 株 Type Ⅰに対して低下（1 年後：86％、2 年後：83％、3 年後：71％）、B 群（sOPV → wIPV → wIPV → wIPV）で Sabin 株 Type Ⅲに対して低下（2 年後：97％）を認めた。他の群、他の株については 100％を維持していた。少数ではあるが、接種後 3 年間で防御レベルを下回る例が生じたことは、我が国における潜在的リスクを反映している可能性がある（福岡、2013 ～ 2018 年、前向き cohort study）。
⑩　水痘ワクチンの公費助成導入により、対象児のワクチン接種率は 81％に達し、特に低年齢児において水痘患者数の減少を認めた。また、ワクチン接種児では水痘の軽症化を認めた（岡山、2012 ～ 2014 年、実態調査）。
⑪　海外渡航者 33 人（平均 36.1 歳、男 17 人）において DTaP-sIPV ワクチン接種の免疫原性を検討したところ、接種前の抗体保有割合はポリオ（Type Ⅰ、Ⅱ、Ⅲ）：88％、100％、76％、百日咳（抗 PT、抗 FHA）：61％、76％、ジフテリア：97％、破傷風：94％であり、接種後には破傷風 97％を除き、総て 100％の抗体保有割合を示した（静岡、2014 ～ 2015 年、前向き cohort study）。

7）費用対効果分科会

①　高齢者に対する肺炎球菌ワクチン接種の助成対象について費用効果分析を行ったところ、現行の「5 歳刻み（65 歳、70 歳、75 歳・・・）で助成を行なう」施策よりも「65 歳～ 80 歳に一括で助成を行なう」あるいは「65 歳以上に一括で助成を行なう」施策の方が、費用効果に優れることが示唆された。
②　高齢者に対する肺炎球菌ワクチン接種の費用効果分析では、「現行 PPSV23 接種ストラテジー」と比較した「PPSV23・PCV13 選択可能接種ストラテジー」の 1QALY 獲得あたりの増分費用は約 37.9 万円であり、定期接種助成対象ワクチンとして PCV13 の導入は費用対効果に優れていることが示唆された。
③　高齢者に対する帯状疱疹ワクチン接種の費用効果分析では、1QALY 獲得あたりの増分費用は 280 万円（65 ～ 84 歳）から 360 万円（80 ～ 84 歳）であった。65 歳以上高齢者に対する帯状疱疹予防接種は費用対効果に優れ、定期予防接種に含める候補として検討する価値がある。

8）微生物検索・病原診断分科会

①　児童養護施設入所者 30 人（7 ～ 19 歳）では、ワクチン株 A/California/7/2009（H1pdm）に対する幾何平均抗体価（GMT）は 122（接種前）→ 403（接種後）、抗体保有割合（sP）は 83 → 97％に上昇し、流行野生株 A/Osaka/52/2014（H1pdm）に対しても GMT：134 → 221、sP：77 → 93％に上昇した。しかし、H3 に関しては、ワクチン株 A/Texas/50/2012 に対する GMT は 122 → 200、sP：93 → 100％を示したものの、流行野生株 A/Osaka/49/2014 に対しては GMT：20 → 27、sP：30 → 50％と十分な抗体レベルには到らなかった（盛岡、2013/14 シーズン、前向き cohort study）。
②　児童養護施設入所者 27 人（9 ～ 19 歳）では、ワクチン株 A/NewYork/39/2015（H3）に対する GMT は 245（接種前）→ 403（接種後）、sP は 100％→ 100％であったが、流行野生株 A/Osaka/18/2015（H3）に対しては GMT：16 → 30、sP：26 → 48％；A/Osaka/16/2015（H3）に対しては GMT：18 → 27、sP：26 → 52％と十分な抗体レベルには到らなかった（盛岡、2014/15 シーズン、前向き cohort study）。

③　過去 4 シーズンにインフルエンザワクチン接種を受けた児童養護施設入所者では、いずれのシーズンも B 型に対する免疫応答は低かった。交差免疫について検討したところ、ビクトリア系統含有ワクチンを接種したシーズンでは山形系統への免疫誘導が示唆されたが、山形系統含有ワクチンを接種したシーズンではビクトリア系統への免疫誘導は明らかでなかった（盛岡、2010/11 ～ 2013/14 シーズン、前向き cohort study）。
④　大阪府における 2014/15 シーズンおよび 2015/16 シーズンのインフルエンザ流行を比較すると、流行規模、流行時期、流行期間に差異を認めた。また、主流行株は全く異なり、インフルエンザの疫学研究が画一的にできないことを示していると考えられた。

9）広報啓発分科会

米国予防接種諮問委員会（US-ACIP）勧告 2014 年度版、2015 年度版、2016 年版を翻訳し、一般財団法人・日本公衆衛生協会より出版した。

はじめに

かつて我が国では、インフルエンザワクチン無効論が台頭した時期があった。最近では、子宮頸がん予防ワクチンの接種後に広範な疼痛や運動障害が発生したことから、積極的勧奨が一時中止されている。このように予防接種を取り巻く環境には、科学的根拠の不足と解明の困難性という障壁が常に横たわっている。

予防接種を健全な形で普及していくためには、ワクチンの有効性や安全性に関する的確な情報を整備蓄積することが必要である。言うまでもなく、有効性や安全性の評価はヒト集団から得られた情報に基づかねばならないが、我が国では実験結果に基づいた推論が独り歩きしている例もある。

ワクチンギャップの克服に向けて大きな前進を遂げつつある現在、ワクチンの有効性・安全性評価に関する分析疫学研究を担う本研究班の役割は大きく、責任は重い。

A. 研究目的

ワクチンを巡る国外および国内の諸課題について、疫学、小児科、呼吸器内科、産婦人科、臨床薬理学、微生物学、医療経済学等の専門家が共同で分析疫学研究に取り組む。

主要課題としては以下の項目があげられる：インフルエンザワクチンの有効性度合いについて、abstract universal statements（要約された普遍的見解）を得る ; 妊婦へのインフルエンザワクチン優先接種（WHO, 2012）の国内制度化について要否を判断するため、妊婦のインフルエンザ健康影響を評価する ; インフルエンザワクチン、百日咳（DTaP）ワクチン、肺炎球菌ワクチン、ロタウイルスワクチンなどについて、免疫原性や有効性、安全性を検討する ; 有効性や関連因子に関する情報を参照して医療経済モデルを構築し、信頼性の高い費用効果的選択肢（公費助成額など）を提示する ; ワクチンの開発・普及に必要な基盤情報として、VPD およびその候補疾患の健康影響を調査する。

インフルエンザワクチンを巡る従来の問題には、予防接種全般に係る課題が集約されているようである。前記の主要課題に関して、研究を継続しつつ研究ネットワークを確立維持することは、予防接種全般に係る緊急な問題に対応できる体制の確立維持につながると考えられる。

B. 研究方法

厚労省意向による特定研究

1）定点モニタリング分科会（分科会長：福島若葉）

多施設共同症例対照研究（test-negative design）により、季節性インフルエンザワクチンの有効性を継続的にモニタリングする。インフルエンザ様疾患で受診した 6 歳未満児を対象とし、PCR 陽性者を症例、陰性者を対照とした。2013/14 シーズンは 1 地域 4 小児科を対象に予備調査を行なった。2014/15 および 2015/16 シーズンは 2 地域 9 小児科に拡大して調査した。

2）妊婦健康影響調査分科会（分科会長:大藤さとこ）

妊婦に対する季節性インフルエンザの健康影響について、大阪府内の産科医療機関に通院中の妊婦 12,838 人を対象に、インフルエンザ関連入院を調査した（self-control methods）。また、同データを使用

し、前向きコーホート研究の手法により妊婦に対するワクチン接種の有効性を検討した。

プロジェクト研究

3）インフルエンザ分科会（分科会長：原めぐみ）

3 価および 4 価不活化インフルエンザワクチンの免疫原性と有効性を検討した。免疫原性は、健常成人（福岡・東京：入江）、高齢者（高知：松下）、糖尿病患者（久留米：井手）、リウマチ患者（福岡：都留）、炎症性腸疾患患者（佐賀：原）、ネフローゼ患者（久留米：田中）、化学療法中の肺癌患者と慢性閉塞性肺疾患患者（千葉：中島）、を対象に調査した。接種前、接種後、流行後に血清を採取し、HI 価を測定。幾何平均抗体価（geometric mean titer: GMT）、平均上昇倍数（mean fold rise: MFR）、抗体保有割合（seroprotection proportion（sP）: HI 価≧ 1:40 の割合）、抗体応答割合（seroresponse proportion（sR）：接種前 HI 価と比し 4 倍以上上昇した者の割合）を算出し、国際基準に則って評価した。有効性については、保育園児（札幌：森）、小学生（土浦：山口）、健常成人（福岡・東京：入江）を対象に、コーホート研究や antibody efficacy の手法を用いて評価した。

4）百日咳分科会（分科会長：岡田賢司）

現行のワクチンプログラムによるワクチン有効性を検討するため、多施設共同症例対照研究を実施した。20 歳未満の百日咳患者を症例とし、同性・同年齢の友人対照 6 人、病院対照 5 人を選定した。解析では、DTaP ワクチンの有効性、および百日咳発症に対するその他の関連因子を検討した。

5）高齢者肺炎分科会（分科会長：鈴木幹三）

高齢者肺炎に対するワクチン予有効性を検討するため、多施設共同症例対照研究を実施した。2009 ～ 2014 年の調査では、65 歳以上の肺炎患者（誤嚥性肺炎は除外）を症例、年齢・性が対応する対照を同科（呼吸器内科）と他科から 2 人選定し、肺炎球菌ワクチンとインフルエンザワクチンの有効性、およびその他の関連因子を検討した。

2014 年 10 月、高齢者に対する肺炎球菌ワクチン接種が定期接種化されたことを受け、先行調査のプロトコールを一部修正し、新規の多施設共同症例対照研究を実施している。65 ～ 90 歳の肺炎患者（誤嚥性肺炎は除外）を症例とし、出生年度・性が対応する対照を 5 人選出している。解析では、肺炎球菌ワクチンとインフルエンザワクチンの有効性、およびその他の関連因子を検討する。

6）新規ワクチン検討分科会（分科会長：中野貴司）

接種普及に関心が高い複数のワクチン（DTaP-IPV、水痘ワクチン、ロタウイルスワクチンなど）について、免疫原性や有効性、安全性を検討するとともに、接種コンプライアンスに関する調査を行った。

また、2011 年の不活化ポリオワクチン導入に先だって行われた「OPV、IPV、DTaP-IPV 互換性試験」の対象者で、接種後の抗体持続を検討した。

7）費用対効果分科会（分科会長：星　淑玲）

1）～ 6）のデータを参照しながら、各種ワクチンを導入した際の費用対効果を医療経済学の立場から検討した。

8）微生物検索・病原診断分科会（分科会長：森川佐依子）

呼吸器系ウイルスの検索、病原診断に関するアドバイスを行う。また、毎シーズン、班員が採取する検体についてインフルエンザウイルスを分離し、確度の高いワクチン有効性研究を可能とした。

さらに、インフルエンザワクチンによって誘導される抗体を、ワクチン株と流行野生株を測定抗原に使用して測定し、perfectly- or imperfectly-matched antibody がワクチンの有効性に及ぼす影響を検討した。

9）広報啓発分科会（分科会長：小笹晃太郎）

米国 CDC と連絡を取りながら、米国予防接種諮問委員会（ACIP）の勧告について、発行時期、注意点や変更点などについての情報を収集した。

若手研究者を中心に同勧告を共同翻訳し、一般財団法人・日本公衆衛生協会より出版した。

（倫理面への配慮）

本研究全般に関して、「博多クリニック臨床試験審査委員会（医療法人相生会博多クリニック院長設置）」の承認を得た。また、研究分担者においても、必要に応じて所属機関の倫理委員会より承認を得た。

C. 主要分科会のまとめ

厚労省意向による特定研究

1）定点モニタリング分科会（分科会長：福島若葉）

我が国の小児におけるインフルエンザワクチンの有効性を継続的にモニタリングすることを目的とした。6 歳未満児を対象に以下の 2 研究を実施した。デザインはいずれも多施設共同症例対照研究（test-negative design）であり、対象者登録時に系統的な手順を踏むことで、選択バイアスを極力回避している。

福島らは、2013/14 シーズンから 3 シーズン連続で研究を実施した。大阪府内あるいは福岡県内の小児科診療所において、各シーズンの流行期にインフルエンザ様疾患（ILI）で受診した 6 歳未満の小児を対象とした（2013/14 シーズン 821 人、平均 2.7 歳 ;2014/15 シーズン 857 人、平均 2.7 歳 ;2015/16 シーズン 914 人、平均 2.9 歳）。real-time RT-PCR（以下、PCR）法による病原診断をアウトカム指標として、検査確定インフルエンザに対するワクチン有効率を算出した。2013/14 シーズン以降 3 シーズンの結果をまとめると、検査確定インフルエンザに対する 1 回接種の有効率は 33％～ 53％、2 回接種の有効率は 50％～ 60％であり、2 回接種の有効率はすべてのシーズンで統計学的に有意であった。型・亜型別に有効率をみると、ワクチン株と流行株の抗原性が良好に合致しているシーズンでは、有効率が 65％程度まで上昇すると考えられた。また、すべてのシーズンで、3～5 歳よりも 1～2 歳で有効率が高かった。

中村らは、2015/16 シーズンに実地診療データに基づく研究を実施した。石川県内の小児科プライマリー診療 13 施設において、流行期に ILI で受診した 2,880 人（平均 3.0 歳）を対象とした。迅速診断キットによる病原診断をアウトカム指標として、検査確定インフルエンザに対するワクチン有効率を算出した。ワクチン有効率は、「（回数にかかわらず）接種あり」で 28％（95％ CI：12％～ 41％）であり、有意な発病防止効果を認めた。A 型に対する有効率は 40％（95％ CI：22％～ 54％）と有意であり、B 型に対しては 22％（95％ CI：2 ～ 39％）と有意ながらも低かった。年齢別有効率は 0 ～ 1 歳で－ 7％、2 ～ 3 歳で 38％、4 ～ 5 歳で 40％であり、0 ～ 1 歳の有効率のみが有意でなかった。

上記 2 研究は、特にサブ解析の結果で異なる点もある。いずれの研究も登録時の選択バイアスを極力回避する工夫を講じているため、妥当性に関する深刻な問題から生じる違いではなく、地域や対象集団の特性、アウトカム指標の精度などの影響を受けた結果かもしれない。

我が国の小児におけるインフルエンザワクチン有効性の論拠が不足している中、本プロジェクトの継続実施により “abstract universal statements（要約された普遍的見解）” を導き、行政施策に活用することができる。なお、test-negative design によるインフルエンザワクチン有効性研究で、迅速診断結果をアウトカム指標としたものには、選択バイアスの影響が否定できない報告（実地臨床の範囲内で安易に行うなど）が我が国で増加していることが懸念される。本プロジェクトで実施した中村らの研究は、堅固なデザインの下に行う模範的研究を示す意味でも重要である。

2）妊婦健康影響調査分科会（分科会長：大藤さとこ）

2012 年 11 月に WHO が示した positioning paper「妊婦を季節性インフルエンザワクチンの最優先接種対象に位置付けるよう推奨する」を受け、大阪産婦人科医会の協力のもと、self-control methods の手法により、我が国の妊婦における「季節性インフルエンザの健康影響」を検討した。妊婦 12,838 人を調査対象として「2010/11 ～ 2013/14 シーズンの呼吸器疾患と関連する入院」を調査した結果、妊娠中（リスク期間）の入院率は 2.54 per 10,000 woman-months であり、非妊娠期（コントロール期間）の入院率（1.08 per 10,000 woman-months）に比べて、$RR_{M\text{-}H}$ で 4.30 倍、高かった（95％ CI：1.96-9.41）。特にインフルエンザ関連の基礎疾患を有する者では、妊娠中に「流行期の呼吸器疾患と関連する入院」のリスク増加が、より顕著となった（$RR_{M\text{-}H}$=6.58、95％ CI：1.58-27.4）。

さらに、同データを用いて、前向きコーホート研究デザインにより、妊婦に対するワクチン接種の有効性を検討した。妊婦に対するワクチン接種の OR（95％ CI）は、自身のインフルエンザ診断に対して 0.77（0.60-0.98）、自身のインフルエンザ関連入院に対して 0.76（0.26-2.21）であり、インフルエンザ診断に対するワクチン有効性には有意差を認めた。また、出生児のインフルエンザ・入院に対する母親のワクチン接種の効果を検討したところ、「出生児のインフルエンザ」に対する「母親のワクチン接種」

のOR（95％CI）は、妊娠中の接種で0.39（0.19-0.84）、出産後の接種で0.47（0.17-1.28）であり、妊娠中の接種によるOR低下には有意差を認めた。「出生児のインフルエンザ入院」に対しても「母親のワクチン接種」のOR（95％CI）は0.27（0.06-1.24）に低下し、境界域の有意差を示した。妊婦に対するインフルエンザワクチン接種は、自身のインフルエンザ予防のみならず、出生児のインフルエンザ・入院の予防にも効果的であると考えられた。

プロジェクト研究

3）インフルエンザ分科会（分科会長：原めぐみ）

健常成人およびハイリスク者を対象として不活化インフルエンザワクチン（IIV）の免疫原性や抗体の推移と、それらの関連要因を明らかにするとともに、ワクチンの有効性を評価した。

入江らは、2014/15、2015/16および2016/17の3シーズンに、IIVを接種した健常成人を対象に先行シーズンのIIV接種や自然感染が、後続シーズンの免疫原性に与える影響を検討したところ、IIV接種後、ワクチン株に対し1：40以上の抗体を保有するものは、抗原類似性に関わらず、感染が防御されること、さらに、感染の有無に関わらず、後続シーズンのsPも有意に高いことを明らかにした。井手らは糖尿病患者、田中らはネフローゼ患者を対象に2014/15シーズンにIIV3を、都留らはリウマチ患者、原らは炎症性腸疾患患者を対象に2015/16シーズンにIIV4を接種し、いずれのハイリスク集団においても良好な免疫原性を確認した。糖尿病患者では高齢群、HbA1c高値群、AGE高値群においてsRが低下傾向を示した。ネフローゼ症候群に使用する程度の量または期間の免疫抑制剤であれば免疫原性に影響はないが、生物学的製剤であるリツキシマブでは、投与後B細胞回復までは免疫原性が得られないことを明らかにした。炎症性腸疾患患者では2回接種による更なる抗体上昇がないことも確認した。山口は小学生を対象として前向きコーホート研究のデザインで有効性を評価し、A型が流行した2014/15シーズンにはA型に対するワクチン有効率44％、混合流行の2015/16シーズンの有効率はそれぞれ、A型33％、B型31％と推計した。

免疫原性の評価では、ワクチン接種前、接種後、流行終了後に血清を採取し、HI価を一括して測定する必要があるため、調査や測定は年度をまたいで行われる。2014/15シーズンには各集団における免疫原性の他、それらに関連する因子などについてより詳細な検討を実施し、2015/16シーズンには主に集団全体での評価を実施した。前向きコーホート研究による有効性評価は2006/07シーズンから継続して実施しているが、同様にシーズンが年度をまたぐため、本研究期間は2014/15、2015/16シーズンの解析を行った。

健常人集団と同様に、糖尿病、ネフローゼ、リウマチ、炎症性腸疾患などのハイリスク集団においてもインフルエンザワクチン接種により有効な免疫原性が得られるが、疾患や治療の状況、接種のタイミングを考慮する必要性が示唆された。また、小児における有効率はおおむね40％前後であることが示された。これらの成果は今後のインフルエンザワクチン接種推奨の際の科学的根拠として活用できる。

4）百日咳分科会（分科会長：岡田賢司）

砂川らは、妊婦への百日咳含有ワクチン接種推奨の観点から、知識・態度とワクチン接種行動に関する調査、および百日咳流行下におけるワクチン有効性に関する疫学研究を行った。妊婦を対象とした調査は、熊本県内1病院の妊婦検診受診者を対象とした。2016年の調査時点では、妊婦の約1/3が百日咳含有ワクチン接種を受けると回答した。接種行動と有意に関連した事項は、（1）有効性が証明され、かつジカウイルス感染症など流行中の疾患であれば接種行動は促進されること、（2）医師からの情報が有効であること、（3）インフルエンザワクチンなど過去の妊娠中のワクチン接種も促進的な要因であった。百日咳流行下における百日咳含有ワクチンの有効性については、平成26年までの高知県内医療機関受診者を対象にした調査で、百日咳ワクチン4回接種後48か月までの有効率は約84％で統計学的に有意であったと報告した。

岡田らは、DTaPワクチンの百日咳に対する予防効果を確認した先行研究と同一の研究計画で、調査地域および年度を追加して、DTaPワクチンの有効性を再検討した。この3年間で対照数の増加により、DTaPワクチンの有効率は94％と算出されただけでなく、先行研究で検出できなかった接種回数別の有効性も確認できた。我が国が世界に先駆け開発・導入したDTaPワクチンの有効性は地域および調査年を変えても確認できた。さらに、ワクチン未接種者

と 4 回接種者を解析対象として、ワクチン最終接種からの期間と百日咳発症との関連を検討した。今回もワクチン接種後の経過年数が長いほど、ワクチン有効性が低くなることはなかった。

5）高齢者肺炎分科会（分科会長：鈴木幹三）

高齢者肺炎に対する肺炎球菌ワクチンとインフルエンザワクチンの有効性、およびその他の発症関連因子を明らかにする。65 歳以上の肺炎患者（誤嚥性肺炎は除外）を症例、性、年齢が対応する対照を呼吸器内科と他科から選定して多施設共同症例対照研究を行い、肺炎球菌ワクチンとインフルエンザワクチンの有効性、および嗜好飲料との関連を検討した。肺炎球菌ワクチン接種、インフルエンザワクチン接種の肺炎予防効果は有意には至らなかったが、肺炎球菌性肺炎に限定すると、肺炎球菌ワクチンの有意な予防効果が検出された（OR=0.23）。コーヒー 1 日 2 杯以上飲用者は、飲まない者に比べて、肺炎に対する OR（0.50）は有意に低下した。

北海道において、肺炎球菌ワクチン接種に関する横断研究を行い、confounding by indication と healthy vaccinee bias を検討した。北海道の 2 つの町において、70 歳から 84 歳までの在宅の住民全員を対象に自記式調査を行った。いずれの町においても、肺炎球菌ワクチン接種者は肺炎の経験がある者が多いなどの confounding by indication が、また、インフルエンザワクチン接種者が多いなどの healthy vaccinee bias が示唆された。

肺炎球菌ワクチン接種により肺炎球菌性肺炎の予防効果を認めたことは、高齢者における市中肺炎予防のため肺炎球菌ワクチンが定期接種化されたことを支持するものである。なお、地域在住高齢者における肺炎球菌ワクチンの有効性評価に際しては、confounding by indication と healthy vaccinee bias に留意する必要がある。

6）新規ワクチン検討分科会（分科会長：中野貴司）

ワクチンは国民の健康を守るための大切な手段であるが、厚生行政の役割として、その有効性と安全性を常に検証し、結果を社会に情報発信する必要がある。本分担研究では、新しく導入や定期接種化された、あるいは今後の普及が期待されるワクチンについて検討することを目的とした。

定期接種ワクチンとして、水痘ワクチン普及による効果の検討、B 型肝炎ワクチンの互換性に関する研究、抗体価持続によるポリオワクチン接種スケジュールの評価を行った。現在定期接種化が検討されているロタウイルスワクチン、国民のニーズがある渡航者ワクチンについても検討した。

水痘ワクチンの定期接種化は大いに患者数を減少させる効果があり、B 型肝炎ワクチンの互換性に関するデータは円滑な定期接種化に貢献できた。中和抗体（NA）価の経時的推移の検討により今後のポリオワクチン接種スケジュールに有用なデータが得られ、ロタウイルスワクチンは疾病制御効果が期待できることがわかった。渡航者ワクチンについては、A 型肝炎ワクチンと四種混合ワクチン（DTaP-IPV）の現場での運用に役立つ結果を報告できた。

ワクチンの定期接種化による効果、接種スケジュールの評価、海外渡航者への感染症対策など様々な観点からワクチンの有効な活用に関する成績を報告することができた。定期接種制度や国民のニーズを受けて実施された本研究により得られた成果は、予防接種施策に対して有用なデータを提供できていると考える。今後、必要な研究を継続するとともに、予防接種の実施にともない発生する費用と得られる効果のバランスも評価し、その適宜性の判断に活用できる研究も実施したい。

7）費用対効果分科会（分科会長：星　淑玲）

高齢者に対する肺炎球菌ワクチン接種の費用対効果、及び水痘ワクチンを用いた帯状疱疹予防接種の費用対効果について分析を行い、自治体が、肺炎球菌ワクチンの接種方式（年齢の選択、ワクチンの選択）や帯状疱疹予防接種の助成導入を決定する際の検討に資することを目的とした。また、定期接種化が議論されているロタウイルスワクチン接種の費用対効果についても検討した。

費用効果分析の手法を用いて分析を行った。各研究は、（1）文献レビュー、（2）ストラテジーの設定、（3）モデルの構築、（4）データの収集と推計、（5）ICER の推定、（6）感度分析の実施、（7）論文のまとめ、の手順に沿って実施した。ICER（増分費用効果比 , incremental cost-effectiveness ratio）、すなわち、追加的に 1QALY（質を調整した生存年 Quality adjusted life year）を獲得するための追加費用を用いて接種の効率性を検討した。

（1）23 価肺炎球菌ワクチンについて。「現行接種ス

トラテジー」、「65 ～ 80 歳年齢限定一括接種ストラテジー」、「65 歳以上一括接種ストラテジー」の順で、それぞれの ICER が 2,911 万円、1,123 万円、1,304 万円であった。高齢者の 23 価肺炎球菌ワクチン接種は、費用対効果に優れるとは言い難いが、「65 歳 ～ 80 歳の年齢限定一括接種ストラテジー」または「65 歳以上一括接種ストラテジー」の方が、現行の分割ストラテジーより費用対効果に優れることが示唆された。

(2) 13 価肺炎球菌ワクチンの導入について。現行 PPSV-23 接種ストラテジーと比較した「PPSV-23・PCV-13 選択可能接種ストラテジー」の ICER は約 37.9 万円であり、定期接種助成対象ワクチンとして PCV-13 の導入は費用対効果に優れることが示唆された。

(3) 国産水痘ワクチンを用いた 65 歳以上高齢者に対する帯状疱疹予防接種について。予防接種プログラムの ICER は約 280 万円（65 ～ 84 歳）から 360 万円（80 ～ 84 歳）であった。高齢者に対する帯状疱疹予防接種は費用対効果に優れ、定期予防接種に将来含める候補として検討する価値があることが示唆された。

(4) 乳幼児に対するロタウイルスワクチンの定期接種化について、ICER は約 688 万円であった。乳幼児に対するロタウイルスワクチン接種は概ね費用効果的と考えられる。

三つの研究はそれぞれ論文にまとめて欧文の学術誌に投稿した。国内外の学術分野や政策分野に情報提供するという目的を達成したと考えられる。

1) Economic Evaluation of Immunisation Programme of 23-Valent Pneumococcal Polysaccharide Vaccine and the Inclusion of 13-Valent Pneumococcal Conjugate Vaccine in the List for Single-Dose Subsidy to the Elderly in Japan. PLoS One. 2015 Oct 7;10 (10):e0139140.
2) Economic evaluation of routine infant rotavirus immunisation programme in Japan. Hum Vaccin Immunother. 2016 (In press)
3) Cost-effectiveness of varicella vaccine against herpes zoster and post-herpetic neuralgia for elderly in Japan. (投稿中)

8) 微生物検索・病原診断分科会（分科会長：森川佐依子）

インフルエンザワクチンの抗体誘導能は、ワクチン株に対して誘導される抗体価を測定し、評価されている。しかしながらワクチンの臨床効果を検討する場合、流行状況や流行期分離株（以下：野生株）に対する抗体誘導能を合わせて検討する必要がある。従って、流行状況の把握、野生株の解析を行うとともに、若年層を対象にワクチン株、野生株を用いた免疫応答を比較した。

2013/14 シーズン、2014/15 シーズンに季節性インフルエンザワクチンの接種を受けた児童養護施設の入所者を対象とした。ワクチン接種は当該シーズン前の 10 月末、採血は接種前または接種時（I）、接種約 1 ヵ月後（II）に行った。血清は、定法に従い処理後、当該シーズンのワクチン株および野生株を用い HI 価を測定した。また、2014/15 シーズン、2015/16 シーズンの流行状況を把握するとともに、野生株の抗原解析、HA 遺伝子の系統樹解析、インフルエンザウイルス以外の呼吸器ウイルスの検出を行った。

2013/14 シーズンの主流行株は AH1pdm09 亜型であり、2009 年の初流行以降抗原性が大きく変化していないため、ワクチン株の A/ カリフォルニア /7/2009 および、野生株 A/ 大阪 /52/2014 に対する GMT、sR、MFR、sP は同等であった。一方、2014/15 シーズンの主流行株は AH3 亜型であり、ワクチン株の A/ ニューヨーク /39/2015 と、野生株 A/ 大阪 /16/2015（サブクレード 3C2a）および野生株 A/ 大阪 /18/2015（サブクレード 3C3a）に対する GMT、sR、MFR、sP は、ワクチン株と野生株とで大きく乖離する結果となった。2014/15 シーズンの AH3 亜型野生株は、ワクチン株である A/ ニューヨーク /39/2015 とは抗原性も異なり、HA 遺伝子の系統樹解析上でも異なるサブクレードに属した。

野生株を測定抗原として用いた検討から、2013/14 シーズンでは、パンデミック時より抗原性が変異していない AH1pdm09 亜型については野生株に対する免疫応答でも国際的な評価基準である EMA 基準を満たす抗体を誘導したことが示された。2014/15 シーズンでは、2 種の異なるサブクレードに属する野生株のいずれに対しても EMA 基準に達していなかったが、sP の指標を HI 価≧ 1：20 とすると、それぞれ A/ 大阪 /18/2015 に対し 85％、A/ 大阪 /16/2015 に対し 96％であった。ワクチン株と野生株に対する免疫応答に乖離はあるが、野生株に対して低い抗体価しか獲得できない状況下での抗体

価と防御の意義、並びにワクチン株選定に由来する問題を再検討する必要があると考えられた。

9）広報啓発分科会（分科会長：小笹晃太郎）

我が国におけるインフルエンザの予防と対策が、世界的な観点で標準的と考えられる手法によって行われるようになることに寄与する。

毎年 8 月頃に米国疾病管理センター（CDC）が刊行する MMWR の分冊または記事として掲載される、米国の予防接種諮問委員会（ACIP）勧告を翻訳して、日本公衆衛生協会より出版した。

本勧告では 2010 年より、月齢 6 ヵ月以上のすべての人々に対する普遍的接種（universal vaccination）を勧奨している。勧告が分冊の場合は、当該シーズンのワクチン推奨株のほか、インフルエンザに関する背景や疫学、インフルエンザワクチンの免疫原性・有効性・安全性、重症インフルエンザ関連合併症のリスクのある人等への接種指針、ワクチンの有害事象やそれに関連する特性を有する人（卵アレルギーを含む）への接種指針、米国で承認されている主要な製剤とその使用上の留意点等が記載されている。勧告が MMWR 内の記事の場合には、上記のうち重要な点が概説されている。上記内容は、本研究期間開始時（H26）頃にはインフルエンザワクチンの有効性評価等の研究結果がほぼ確定されていたため、本研究期間（H26-28）においては、大きな変化はみられなかった。ただし、2009 年に出現した新型インフルエンザがその後に季節性インフルエンザとして定着したことにより、ワクチン接種歴に基づいた小児への接種指針の変化があったこと、弱毒化生ワクチンの有効性の評価が変動したこと、卵アレルギーの人に関する接種方法が変更されたことなど、記述内容に若干の変化がみられた。

成果物（翻訳書）は日本公衆衛生協会より出版され、インフルエンザワクチン接種に関係する医療機関や保健活動関連機関が参考とすることにより、知識の普及が図られる。

D. 研究結果と考察

厚労省意向による特定研究

1）定点モニタリング分科会

① 福島、加瀬らは、インフルエンザワクチンの有効性を継続的にモニタリングするため、多施設共同症例対照研究（test-negative design）を実施した（2013/14 シーズン、症例対照研究）。大阪府下の小児科診療所 4 施設において、流行期間中（定点あたり患者数 5 人以上の期間と定義：2014 年 1 月 20 日～ 3 月 30 日）にインフルエンザ様疾患（ILI）で受診した 6 歳未満の小児 821 人（平均 2.7 歳、男 458 人）を対象とした。鼻汁吸引検体を用いて real-time RT-PCR 法による病原診断を行い、インフルエンザウイルス陽性の者を症例、インフルエンザウイルス陰性の者を対照（test-negative control）とした。調査シーズンのインフルエンザワクチン接種に関する情報は、診療録あるいは母子健康手帳から転記した。症例と対照のワクチン接種率を比較し、多重ロジスティック回帰モデルにより PCR 陽性インフルエンザに対するワクチン有効率（VE）を（1 − OR）× 100％により算出した。PCR 陽性インフルエンザは 386 人であり、PCR 陽性インフルエンザに対するワクチン接種の調整 OR は、1 回接種で 0.47（95％ CI：0.26-0.85）、2 回接種で 0.49（0.32-0.77）であった。年齢階級別にみると、若年層で顕著な OR 低下を認めた（2 回接種の OR は、1 ～ 2 歳で 0.45、3 ～ 5 歳で 0.87）。

また、2014/15 シーズンは、大阪府と福岡県の 2 地域で、同内容の調査を実施した（2014/15 シーズン、症例対照研究）。大阪府・福岡県の小児科診療所 9 施設において、流行期間中に ILI で受診した 6 歳未満の小児 857 人（平均 2.7 歳、男 459 人）を解析対象とした。PCR 陽性インフルエンザは 302 人であり、PCR 陽性インフルエンザに対するワクチン接種の調整 OR は、1 回接種で 0.59（0.33-1.07）、2 回接種で 0.50（0.31-0.81）であった。年齢階級別にみると、若年層で顕著な OR 低下を認めた（2 回接種の OR は、1 ～ 2 歳で 0.41、3 ～ 5 歳で 0.62）。

さらに、2015/16 シーズンも、大阪府と福岡県の 2 地域で、同内容の調査を実施した（2015/16 シーズン、症例対照研究）。大阪府内あるいは福岡県内の小児科診療所 9 施設において、流行期間中に ILI で受診した 6 歳未満の小児 914 人（平均 2.9 歳、男 487 人）を解析対象とした。PCR 陽性インフルエンザは 424 人であり、PCR 陽性インフルエンザに対するワクチン接種の調整 OR は、1 回接種で 0.67（0.36-1.24）、2 回接種で 0.40（0.26-0.60）であった。年齢階級別にみると、若年層で顕著な OR 低下を認めた（2 回接種の OR は、1 ～ 2 歳で 0.33、3 ～ 5 歳で 0.46）。

別途、中村、福島は、石川県内の 13 医療機関の小児科外来において、インフルエンザワクチンの有効性を検討するため、多施設共同症例対照研究（test-negative design）を実施した（2015/16 シーズン、症例対照研究）。流行期間中（定点あたり患者数 5 人以上の期間と定義）に ILI で受診した生後 9 ヵ月から 6 歳未満の小児 2,880 人（平均 3.0 歳）を解析対象とした。鼻腔拭い液または鼻汁検体を採取し、インフルエンザ抗原検出用診断キットによる検査を行い、陽性者を症例、陰性者を対照（test-negative control）とした。調査シーズンのインフルエンザワクチン接種に関する情報は、診療録あるいは母子健康手帳から転記した。症例と対照のワクチン接種率を比較し、多重ロジスティック回帰モデルにより迅速診断キット陽性インフルエンザに対するワクチン有効率（VE）を（1 − OR）× 100％により算出した。モデルには、年齢、就園の有無、同胞の有無、昨シーズンのインフルエンザ罹患歴、今シーズンのインフルエンザ罹患歴、発症週数、発症から診断までの日数、診断時までの最高体温、昨シーズンのワクチン接種歴、今シーズンのワクチン接種状況を含めた。迅速診断キット陽性インフルエンザは 1,444 人で、うち A 型が 511 人であった。迅速診断キット陽性インフルエンザに対するワクチン接種（1 回以上）の VE は 28％（12-41％）、A 型に対する VE は 40％（22-54％）、B 型に対する VE は 22％（2-39％）であり、いずれも有意なワクチン有効性を示した。年齢別では、0 ～ 1 歳児における VE は −7％であり有効性を認めなかったが、2 ～ 3 歳児では 38％、4 ～ 5 歳児では 40％と有意なワクチン有効性を示した。また、2 ～ 5 歳児で、今季のみ接種と 2 季連続接種の VE を比較したところ、今季のみ接種の VE は 27％、2 季連続接種の VE は 40％であり、2 季連続接種の VE は有意差を認めた。

2）妊婦健康影響調査分科会

① 出口、浦江、大藤らは、2012 年 11 月に WHO が示した positioning paper「妊婦を季節性インフルエンザワクチンの最優先接種対象に位置付けるよう推奨する」を受け、大阪産婦人科医会と共同で、妊婦における「季節性インフルエンザの健康影響」を調査した（2010/11 ～ 2013/14 シーズン、self-control methods）。2013/14 シーズン開始前（2013 年 10 月～ 12 月）に、大阪府下の産科医療機関に通院していた妊婦 12,838 人を調査対象とした。登録時（2013 年 10 月～ 12 月）に、「過去 3 年間」の入院歴に関する情報を収集し、2013/14 シーズンの流行が終息した 2014 年 5 月に、2013/14 シーズンの入院に関する情報を収集した。解析では、流行期を「定点報告数 5 以上の期間」、結果指標を「流行期の呼吸器疾患と関連する入院」と定義し、2010/11 ～ 2013/14 シーズンにおける「妊娠中（リスク期間）」の入院率が、「非妊娠期（コントロール期間）」の入院率に比べて、何倍高くなるか（$RR_{M\text{-}H}$、調整相対危険）を算出した。2010/11 ～ 2013/14 の 4 シーズンに「流行期の呼吸器疾患と関連する入院」があったのは合計 25 人で、うち 9 人は妊娠中の入院であった。妊娠中の当該入院率は 2.54 per 10,000 woman-months であり、非妊娠期の入院率（1.08 per 10,000 woman-months）に比べて、$RR_{M\text{-}H}$ で 4.30 倍、高かった（95％ CI：1.96-9.41）。特にインフルエンザ関連の基礎疾患を有する者では、妊娠中に「流行期の呼吸器疾患と関連する入院」のリスク増加が、より顕著となった（$RR_{M\text{-}H}$=6.58、95％ CI：1.58-27.4）。

また、同調査のデータを用いて、妊婦に対するインフルエンザワクチンの有効性を検討した（2013/14 シーズン、前向き cohort study）。2013 年 9 月～ 2014 年 1 月に、大阪府下の産科医療機関に通院していた妊婦のうち、2013/14 シーズン開始時に妊娠していた者 8,472 人を解析対象とした。2013/14 シーズン中に、インフルエンザの診断を受けた者は 339 人（4％）、インフルエンザ関連で入院した者は 17 人（0.2％）であった。ワクチン接種の OR（95％ CI）は、インフルエンザ診断に対して 0.77（0.60-0.98）、インフルエンザ関連入院に対して 0.76（0.26-2.21）であった。一方、2013/14 シーズン開始前に出生した児 3,441 人を対象に、出生児のインフルエンザ・入院に対する母親のワクチン接種の効果を検討したところ、「出生児のインフルエンザ」に対する「母親のワクチン接種」の OR（95％ CI）は、妊娠中の接種で 0.39（0.19-0.84）、出産後の接種で 0.47（0.17-1.28）であり、妊娠中の接種による OR 低下は統計学的に有意であった。「出生児のインフルエンザ入院」に対しても「母親のワクチン接種」の OR（95％ CI）は 0.27（0.06-1.24）に低下し、境界域の有意差を示した。妊婦に対するインフルエンザワクチン接種は、自身のインフルエンザ予防のみならず、出生児のインフルエンザ・入院の予防にも効果

的である。妊婦は、出生児のインフルエンザを予防するためにも、インフルエンザワクチンを接種すべきであるし、妊娠中にワクチン接種を受ける機会を逃した場合には出産後の接種も効果的であることが示唆された。

プロジェクト研究

3）インフルエンザ分科会

① 森らは、札幌市の保育園児を対象に、インフルエンザワクチンの有効性を研究した（2011/12 ～ 2013/14 シーズン、後ろ向き cohort study）。2011/12 シーズンの調査では、2012 年 5 月に札幌市内の 10 保育所に通っていた園児 629 人を対象とし、2011/12 シーズンのインフルエンザワクチン接種（母子手帳より転記）およびインフルエンザ診断の情報を収集した。2012/13 シーズンの調査では、2011/12 シーズン調査に協力が得られた 629 人に対し、2013 年 5 月に調査票を送付し、2012/13 シーズンのワクチン接種状況、およびインフルエンザ診断の情報を収集した（回答者 588 人）。2013/14 シーズンの調査では、2014 年 5 月に調査票を送付し、2013/14 シーズンの情報を得た（回答者 572 人）。各シーズンの 10 月 1 日を観察開始日とし、インフルエンザ罹患日あるいは 4 月 30 日までを観察期間とした。ワクチン接種（1 回以上）のインフルエンザ診断に対する調整ハザード比（HR）は、2011/12 シーズン：0.73（95% CI, 0.53-0.99）、2012/13 シーズン：0.40（0.22-0.71）、2013/14 シーズン：0.74（0.52-1.07）であった。年齢別に見ると、1 歳～ 3 歳までの低年齢層では有効差を検出したが、4 歳以降ではいずれのシーズンも有意な HR の低下を認めなかった。本研究ではワクチン接種に関する情報を母子手帳から転記しているため、ワクチン接種に関する情報の精度が高い。しかし、インフルエンザ診断に関しては、医療機関への受診行動に影響を受けている可能性を否定できない。

② 入江、都留らは、医療法人（東京・福岡の 3 施設）の職員を対象に、インフルエンザワクチン毎年接種の免疫応答への影響を検討した（2014/15 ～ 2016/17 シーズン、前向き cohort study）。登録時に、年齢、性、ワクチン接種歴、既往歴、家族数などの背景因子の情報を収集した。また、対象者にインフルエンザワクチンを接種し、接種前、接種 4 週後、流行後の HI 価を測定した。接種後 48 時間の副反応については、自記式質問票で情報収集を行った。更に、流行期間中のインフルエンザ症状、医療機関受診、診断、入院などの発病調査を行い、臨床的有効性についても検討を行なった。

2014/15 シーズンの調査では、156 人（男 56 人、女 100 人）を登録した。接種前の HI 価が 1:40 未満の者は、H1：37％、H3：62％、B：14％であり、うち半数は接種後に HI 価 1:40 以上を獲得した（H1：53％、H3：61％、B：50％）。特に年齢が高い者や接種前 HI 価が低い者では、接種後に HI 価≧ 1:40 を獲得しにくい傾向にあった。有効性に関しては、antibody efficacy の手法、すなわちワクチン接種後に HI 価≧ 1:40 の者の発病率を HI 価 <1:40 の者と比較し OR を算出した（antibody efficacy［％］=（1 － OR）× 100）。また、ワクチン有効率（VE）を「antibody efficacy × 達成率（接種前 HI 価が <1:40 であった者のうち接種後に 1:40 以上を獲得した者の割合）」により算出した。なお、当該シーズンの主流行株は H3 であり、分離株の 92％を占めていた。H3 に対して接種後 HI 価≧ 1:40 を獲得した者では、「流行期の有熱性呼吸器疾患」や「迅速診断陽性 A 型インフルエンザ」が少なく、調整 OR（95% CI）はそれぞれ 0.6（0.2-1.8）、0.3（0.1-1.4）を示した（antibody efficacy はそれぞれ 40％と 70％）。また、H3 に対する達成率は 61％であったため、ワクチン有効率は「流行期の有熱性呼吸器疾患」に対して 25％、「迅速診断陽性 A 型インフルエンザ」に対して 42％と算出された。

また、2014/15 ～ 2015/16 シーズンの 2 シーズン連続してワクチン接種を受けた 25 ～ 66 歳の健康成人 141 人（男 59 人、女 82 人）を対象に、インフルエンザワクチン毎年接種の免疫応答への影響を検討した（2014/15 ～ 2015/16 シーズン、前向き cohort study）。対象者にインフルエンザワクチンを 1 回接種し（2014/15 シーズンは 3 価、2015/16 シーズンは 4 価）、接種前、接種 4 週後、流行後の HI 価を測定した。1 シーズン目に接種後 HI 価が 1:40 以上を示した者は、H1：82％、H3：77％、B（山形）：93％、2 シーズン目に接種後 HI 価が 1:40 以上を示した者は、H1：81％、H3：89％、B（山形）：42％、B（ビクトリア）：50％であった。1 シーズン目に接種後 HI 価 1:40 以上を示した者は、1:40 未満の者に比べて、2 シーズン目の接種後にも HI 価 1:40 以上を有する割合が有意に高く（H1：92％ vs. 28％、

H3：94% vs. 73%）、多変量解析による調整OR（95% CI）はH1：2.5（1.3-5.0）、H3：2.2（1.2-3.9）を示した。B型に対しても同様の傾向があり、1シーズン目にB（山形）に対する接種後HI価1:40以上を示した者では、1:40未満の者に比べて、2シーズン目の接種後HI価1:40以上を有する割合が有意に高かった（B（山形）：45% vs. 0%、B（ビクトリア）：53% vs. 10%）。

③　都留らは、福岡県の1医療機関に定期通院中のリウマチ性疾患患者151人を対象に、インフルエンザワクチンの免疫原性を検討した（2015/16シーズン、前向きcohort study）。151人のうち、MTX投与患者は107人、生物学的製剤投与者は60人、PSL投与患者は56人である。対象者にインフルエンザワクチンを1回接種し、接種前（S0）、接種4週後（任意）（S1）、接種8週後（S2）のHI価を測定した。抗体保有率（sP）はH1：46%（S0）⇒84%（S1）⇒78%（S2）、H3：23%⇒73%⇒69%、B（山形）：40%⇒73%⇒70%、B（ビクトリア）：32%⇒69%⇒65%であり、リウマチ性疾患患者におけるインフルエンザワクチンの免疫原性は良好であった。現在、治療内容による免疫原性についても、検討を進めている。

④　井手らは、福岡県の1医療機関に定期通院中の糖尿病患者55人（年齢中央値65.5歳、男25人、Ⅰ型糖尿病：10人）を対象に、インフルエンザワクチンの免疫原性を検討した（2014/15シーズン、前向きcohort study）。対象者の背景因子（糖尿病の病型、合併症、治療状況など）については、診療録から情報を得た。対象者にインフルエンザワクチンを1回接種し、接種前、接種4週後、流行後のHI価を測定した。接種後のsPはH1：76%、H3：76%、B：71%、sRはH1：65%、H3：73%、B：42%であり、糖尿病患者におけるインフルエンザワクチンの免疫原性は良好であった。しかし、背景因子を考慮した多変量解析の結果、肥満者（BMI ≧ 25.0kg/m^2）ではsRが良好である一方で、65歳以上高齢者、HbA1c高値、AGE高値の者では有意ではないもののsRが低い傾向を認めた。

⑤　原らは、佐賀県の1医療機関で、炎症性腸疾患患者141人（平均42.7歳、男80人、クローン病47人、潰瘍性大腸炎94人）および健常成人29人を対象に、インフルエンザワクチンの免疫原性を検討した（2015/16シーズン、無作為化非盲検対照並行群間比較試験）。対象者の生年月日により、ワクチン1回接種群、2回接種群に無作為割付を行い（奇数日生まれは2回接種）、接種前、接種後、流行後のHI価を測定した。1回接種群と2回接種群で、性別、年齢、原疾患の分布に有意差を認めず、無作為割付は適正であったと考えられる。IBD患者と健常成人の免疫原性は同様の傾向を示し、1回接種でいずれのワクチン株についても国際基準を満たす良好な抗体応答が得られた。IBD患者におけるワクチン接種後のsPは、H1N1に対して67%（1回接種群）と63%（2回接種群）、H3N2に対して74%（1回接種群）と80%（2回接種群）、B（プーケット）に対して81%（1回接種群）と86%（2回接種群）、B（テキサス）に対して83%（1回接種群）と82%（2回接種群）であり、2回接種による更なる抗体価の上昇は認めなかった。

⑥　田中らは、福岡県の1医療機関を定期通院中のネフローゼ患者49人（年齢3～24歳、免疫抑制剤使用患者40人、RTX投与患者9人）および健常成人71人を対象に、インフルエンザワクチンの免疫原性を検討した（2014/15シーズン、前向きcohort study）。13歳未満の者にはワクチンを2回接種し、13歳以上の者にはワクチンを1回接種した。接種前、接種4週後、8週後、12週後（2回接種群のみ）、6ヵ月後に採血を行い、HI価の測定を行った。なお、RTX投与患者における免疫原性は、RTX投与によりB細胞が枯渇した状態でワクチン接種を行った群（B細胞枯渇時ワクチン接種群）と、ワクチン接種の1ヵ月後にRTX投与を行った群（ワクチン投与後B細胞枯渇群）との比較を行った。ワクチン投与後B細胞枯渇群は全て13歳以上であった。13歳以上における抗体陽転率は、健常者（8-20%）、免疫抑制剤使用者（4-35%）、ワクチン投与後B細胞枯渇群（25-50%）で同様であったが、B細胞枯渇時ワクチン接種群では0%と低かった。接種後のGMTについても、健常者（58-217）、免疫抑制剤使用者（50-223）、ワクチン投与後B細胞枯渇群（50-202）で同様であったが、B細胞枯渇時ワクチン接種群では40-80と低かった。sPは、健常者77-100%、免疫抑制剤使用者65-100%、ワクチン投与後B細胞枯渇群75-100%、B細胞枯渇時ワクチン接種群100%であった。RTX投与者では、RTX投与1ヵ月前のワクチン接種によりRTXの影響を受けずに抗体陽転および抗体上昇が得られる可能性がある。

⑦ 中島らは、千葉県の医療機関で、呼吸器疾患患者 51 人（平均 69.4 歳、男 41 人）を対象に、インフルエンザワクチンの免疫原性を検討した（2013/14 シーズン、前向き cohort study）。対象の内訳は、化学療法中の肺がん患者 25 人、慢性閉塞性肺疾患（COPD）患者 26 人である。対象者にワクチンを 1 回接種し、接種前（S0）、接種 4 ～ 6 週後（S1）、シーズン後（S2）の HI 価を測定した。接種後（S1）の sP は、肺がん患者では H1：84％、H3：84％、B：65％、COPD 患者では H1：81％、H3：96％、B：92％であった。年齢、性、接種前抗体価で補正し、多変量解析を行なった結果、COPD 患者と比較した肺がん患者の sP に対する OR（95％ CI）は、H1：3.09（0.43-22.1）、H3：0.31（0.02-4.12）、B：0.19（0.02-1.67）であり、化学療法中の肺がん患者の免疫原性は COPD 患者と比べて有意差を認めなかった。

⑧ 松下は、高知県のへき地に在住する 61 歳以上の高齢者 109 人（平均 76.7 歳、男 39 人、基礎疾患あり 22 人）を対象に、2 シーズン連続してインフルエンザワクチンを 2 回接種した場合の免疫原性を検討した（2012/13 ～ 2013/14 シーズン、前向き cohort study）。対象者に不活化インフルエンザワクチンを 4 週間隔で 2 回接種し、接種前、接種 4 週後、接種 22 週後の HI 価を測定した。2 シーズンともに、すべてのワクチン株に対する HI 価は接種 4 週後に有意に上昇し、接種 22 週後に有意に低下した。H1 に対する HI 価は 2 シーズン間で同様であったが、H3 に対する HI 価は 2 シーズン目の方が高かった。B については、2 シーズン目の接種前 HI 価は 1 シーズン目よりも高値を示したが、接種 4 週後、22 週後の抗体応答は低かった。年齢（76 歳未満、76 歳以上）で層化したところ、1 シーズン目接種前の H1 に対する HI 価は 2 群間で差を認めなかったが、接種 4 週後以降の HI 価は、76 歳未満の者より 76 歳以上の者の方が低かった。H3 に対しては、両シーズンとも 2 群間で HI 価に差を認めなかったが、接種 4 週後から接種 22 週後にかけての HI 価の有意な低下は 76 歳以上の者でのみ観察され、76 歳未満の者では認めなかった。B では 1 シーズン目の HI 価は 2 群間で差を認めなかったが、接種 22 週後の HI 価は両群ともに 4 週後から維持されていた。

⑨ 山口らは、茨城県土浦市の小学生（4 校：2,223 人）を対象に、インフルエンザワクチンの有効性を検討した（2014/15 シーズン、前向き cohort study）。2015 年 1 月上旬に基礎調査を行い、年齢、性別、兄弟姉妹数、基礎疾患の有無、インフルエンザワクチン接種歴、罹患歴、等の情報を収集した。また、2015 年 1 月から 3 月の追跡期間中、インフルエンザに罹患した場合は、学校に届け出る欠席報告書と一緒に、本研究用のアンケート（発熱時期、インフルエンザの型、抗ウイルス薬処方等）を提出するよう依頼した。解析では、ワクチン接種回数が 1 回のみの児童はワクチン接種群に入れて検討した。1 回以上ワクチンを接種したと回答したのは 1,198 人（接種率 55％）であった。4 校全体の A 型インフルエンザの発病率は 22％であり、ワクチン有効率（95％ CI）は 44％（24-58％）であった。学年（年齢）が 1 年（歳）上昇する毎に A 型発症のリスクは 0.90 に低下したが、昨年度のインフルエンザ罹患は A 型発症のリスクを 2.04 に上昇させていた。B 型に関しては、発病者が少なく（15 人、0.7％）、ワクチン有効率および発病の関連因子を検討することが困難であった。A 型インフルエンザ罹患児に限定してワクチン接種歴と有熱期間との関連を検討したところ、ワクチン接種者では非接種者と比べて有熱期間が短い傾向にあった（55.4 時間 vs. 62.3 時間、P=0.003）。

2015/16 シーズンにも、茨城県土浦市の小学生（4 校：2,278 人）を対象に、同内容の調査を実施した（2015/16 シーズン、前向き cohort study）。1 回以上ワクチンを接種したと回答したのは 1,135 人（接種率 53％）であった。4 校全体の A 型インフルエンザの発病率は 14％、B 型インフルエンザの発病率は 13％であり、ワクチン有効率（95％ CI）は A 型インフルエンザに対して 33％（1-55％）、B 型インフルエンザに対して 31％（－2-54％）であった。学年（年齢）が 1 年（歳）上昇する毎に A 型発症のリスクは 0.90 倍、B 型発症のリスクは 0.81 倍に低下し、統計学的に有意差を示した。有熱期間は、A 型、B 型ともにワクチン接種群と非接種群の間で有意差を認めなかった。

4）百日咳分科会

① 岡田らは、20 歳未満の百日咳患者を症例とし、性・年齢が同一の「友人対照 6 人」および「病院対照 5 人」を対照とした多施設共同症例対照研究において、現行の DTaP ワクチンの有効性および百日咳発症関連因子を検討した（2009 ～ 2012 年、症例対

照研究）。解析対象は、症例 55 人、対照 90 人（友人対照 69 人、病院対照 21 人）。百日咳発症に対する DTaP ワクチン（1 回以上）の調整 OR（95％ CI）は、0.20（0.04-0.97）で、統計学的に有意差を認めた。接種回数別では、1 ～ 3 回接種で 0.15（0.02-1.24）、4 回接種で 0.22（0.04-1.05）を示した。マッチドペア 101 人（症例 33 人、対照 68 人）に限定した conditional model による解析においても、OR はほとんど変わらなかった。非接種者と 4 回接種者を解析対象として、DTaP ワクチン最終接種からの経過年数による影響を検討したところ、非接種者と比較したワクチン 4 回接種者の OR は、接種後 5.8 年未満の者で 0.24、5.8 ～ 9.1 年経過した者で 0.14、9.2 年以上経過した者で 0.11 に低下しており、「ワクチン接種後の経過期間が長いほど、ワクチン有効性が低い」という関連は認めなかった。

2014 年度以降には、地域（7 医療機関）を追加し、同内容の症例対照研究を実施した。2012 年 4 月～ 2016 年 11 月までに登録された症例 38 人、対照 135 人（友人対照 37 人、病院対照 98 人）の解析では、百日咳発症に対する DTaP ワクチン（1 回以上）の調整 OR（95％ CI）は 0.06（0.007-0.46）であり、接種回数別にみると 1 ～ 3 回接種で 0.04（0.003-0.54）、4 回接種で 0.07（0.006-0.78）という結果を得た。その他の関連因子として、喘息あり（OR=3.84）、母親の妊娠中の喫煙歴（OR=3.98）、周囲の咳患者（OR=3.27）が挙げられた。友人対照は乳児の選出が困難であることが多いが、百日咳への曝露機会などの背景因子が症例と同様であるために、ワクチン有効性を検出しやすい。一方、病院対照は年長児の選出が困難であること、および背景因子が症例と異なるために他因子の影響が大きくワクチン有効性を検出しにくいことが考えられた。しかし、最終的に全対照と症例で比較することでワクチン有効性も関連因子も検出することが可能となった。また、年齢をマッチさせた症例対照研究で、ワクチン接種後の経過年数によるワクチン有効性の減弱の可能性について検討することは困難であり、他の方法での検討が必要と考えられた。

② 砂川らは、高知県の 3 医療機関で、15 歳未満児を対象に、DTaP ワクチンの有効性を検討した（2012 年、症例対照研究）。症状から百日咳が疑われ、LAMP 法による検査診断を受けた 318 人を対象とし、陽性者 102 人を症例、陰性者 216 人を対照（test-negative control）とした。DTaP ワクチン接種の粗 OR（95 % CI）は、1 ～ 2 回接種で 0.15（0.03-0.70）、3 回接種で 0.13（0.03-0.55）、4 回接種で 0.15（0.04-0.57）であった。4 回接種後の経過時間との関連を検討したところ、医療機関で調整後の OR（95% CI）は、接種後 12 ～ 23 ヵ月で 0.05（0.01-0.26）、24 ～ 35 ヵ月で 0.06（0.01-0.40）、36 ～ 47 ヵ 月で 0.15（0.03-0.83）、48 ～ 59 ヵ月で 0.22（0.04-1.23）、60 ヵ月以上で 0.20（0.05-0.82）となった。4 回接種後のワクチン有効性は、徐々に低下するものの接種後 47 ヵ月までは 85%の有効性を示した。ただし、本研究には、ワクチン接種に関する思い出しバイアス、検体採取時期に関する情報不足、交絡因子の存在、百日咳ワクチンの有効性研究として test-negative design を適用することの妥当性などの Limitation がある。

別途、2016 年 9 月から 12 月の期間に熊本県の 1 医療機関を受診した妊婦 987 人を対象に、百日咳含有ワクチン接種に関する意識調査を実施した（2016 年、横断研究）。自記式質問票により、百日咳含有ワクチン接種の意向、ワクチンや疾患に関する知識、態度に関する情報を得た。有効回答 792 人のうち、妊娠中に百日咳含有ワクチンが接種可能なら「接種する」と回答した者は 225 人（28%）であった。接種の意向に関連する項目は、妊婦への百日咳ワクチンが「必要と思う」、「怖くない」、「効果あると思う」、「出生児への予防効果があると思う」、「出生児への副反応を生じさせると思わない」、「妊娠中に季節性インフルエンザワクチンを受けた」、「ジカウイルスに有効なワクチンがあれば接種を希望する」、「ワクチンに関する最も信頼する情報源が医師」であった。これらの情報は、妊婦への百日咳ワクチン接種を検討する際の貴重な情報となることが期待される。

5）高齢者肺炎分科会（肺炎球菌ワクチン）

① 鈴木らは、高齢者肺炎に対するインフルエンザワクチンと肺炎球菌ワクチンの有効性を検討するため、多施設共同・症例対照研究を実施した（2009 ～ 2014 年、症例対照研究）。症例は協力医療機関において新たに肺炎と診断された 65 歳以上の患者である。対照は、症例と性・年齢・外来受診日が対応する同一機関受診患者とし、1 症例につき 2 対照（呼吸器科 1 人、呼吸器科以外の診療科 1 人）を選定した。2009/10 シーズンは A（H1N1）pdm09 が発生したため、2009/10 シーズンは除外し、2010 年 10 月

～ 2014 年 9 月に登録された 469 人（症例 161 人、対照 308 人）を解析対象とした。肺炎に対するワクチン接種の調整 OR（95％ CI）は、インフルエンザワクチン 0.79（0.50-1.25）、肺炎球菌ワクチン 0.76（0.44-1.32）であった。接種パターン別の検討では、両ワクチンとも非接種と比較した調整 OR は、インフルエンザワクチンのみ接種 0.76（0.46-1.27）、肺炎球菌ワクチンのみ接種 0.69（0.30-1.59）、両ワクチンとも接種 0.62（0.32-1.23）であった。また、肺炎球菌性肺炎に対するワクチン接種の調整 OR は、インフルエンザワクチン 0.65（0.31-1.36）、肺炎球菌ワクチン 0.23（0.08-0.66）となり、肺炎球菌ワクチン接種の調整 OR は有意に低下した。

また、2014 年 10 月、高齢者に対する肺炎球菌ワクチン接種が定期接種化されたことを受けて、プロトコールを一部修正し、29 施設の協力を得て、新規の多施設共同症例対照研究を実施している（2016 年 10 月～、症例対照研究）。症例は協力医療機関において新たに肺炎と診断された 65 ～ 90 歳の患者である。対照は、症例と性・出生年度・外来受診日が対応する同一機関受診患者とし、1 症例につき 5 対照を選定している。2017 年 1 月時点で、症例 16 人、対照 80 人を登録している。

別途、近藤、鈴木らは、症例対照研究で得られた情報を詳細に解析することにより、嗜好飲料と肺炎との関連を検討した（2009 年 10 月～ 2014 年 9 月、症例対照研究）。症例は新たに肺炎と診断された 65 歳以上の患者、対照は症例と性・年齢（5 歳階級）・外来受診日が対応する同一機関受診患者 2 人である。医師記入用調査票および患者記入用調査票により、ワクチン接種（肺炎球菌、インフルエンザ）、BMI、基礎疾患、ADL、6 歳以下の小児との同居、喫煙・飲酒習慣、嗜好飲料の情報を得た。症例 199 人、対照 369 人を対象とした多変量解析の結果、1 日 2 杯以上のコーヒー摂取者では、肺炎に対する調整 OR が有意に低下した（OR=0.50、95％ CI：0.28-0.88）。コーヒー摂取による肺炎の OR 低下は、喫煙習慣、年齢、ワクチン接種状況、基礎疾患、にかかわらず、同様であった。これまでの研究においてもコーヒー摂取による呼吸器疾患死亡の低下が示唆されており、高齢者肺炎に対するワクチン有効性を検討する上で考慮すべき要因になりうると考えられる。

② 森らは、北海道の 70 歳～ 79 歳の在宅高齢者 567 人を対象に、肺炎球菌ワクチン接種行動を調査した（2013 ～ 2015 年、横断研究）。在宅で生活する 70 歳～ 79 歳の全町民 567 人を対象とし、2013 年 2 ～ 3 月、2014 年 2 ～ 3 月、2015 年 2 ～ 3 月に合計 3 回の自記式質問票調査を実施した。調査項目は、肺炎球菌ワクチン接種、肺炎既往、インフルエンザワクチン接種、インフルエンザ罹患、過去 1 年間の入院、通院、介護予防事業への参加などである。2013 年調査には 378 人、2014 年調査には 299 人、2015 年調査には 295 人が参加した。肺炎球菌ワクチン接種率は、2013 年：6％、2014 年：7％、2015 年：17％であり、2014 年 10 月には PPSV23 が定期接種の対象となったため、2015 年の接種率が上昇した。肺炎球菌ワクチン接種者は、肺炎既往（2013 年、2014 年調査）、インフルエンザ罹患（2013 年調査）、過去 1 年間の入院歴（2014 年調査）、過去 1 年間の通院歴（2013 年、2014 年調査）を有する者が多く、confounding by indication の存在が示唆された。また、肺炎球菌ワクチン接種者は、インフルエンザワクチン接種者（2013 年、2014 年、2015 年調査）、介護予防事業参加者（2013 年、2015 年調査）が多く、healthy vaccinee bias の存在が示唆された。2015 年調査では、肺炎球菌ワクチン接種における healthy vaccinee bias のみが観察され、confounding by indication は観察されなかったことは、定期接種化に伴う影響と考えられた。

また、北海道の 70 歳～ 84 歳の在宅高齢者 978 人を対象に、肺炎球菌ワクチン接種行動を調査した（2014 ～ 2016 年、横断研究）。在宅で生活する 70 歳～ 84 歳の全町民 978 人を対象とし、2014 年 3 月、2015 年 3 月、2016 年 3 月に合計 3 回の自記式質問票調査を実施した。調査項目は、肺炎球菌ワクチン接種、肺炎既往、インフルエンザワクチン接種、インフルエンザ罹患、過去 1 年間の入院、通院、介護予防事業への参加などである。2014 年調査には 546 人、2015 年調査には 482 人、2016 年調査には 456 人が参加した。肺炎球菌ワクチン接種率は、2014 年：12％、2015 年：22％、2016 年：26％であり、2014 年 10 月に PPSV23 が定期接種の対象となったことにより、接種率は上昇傾向にある。肺炎球菌ワクチン接種者は、肺炎既往（2015 年調査）、インフルエンザ罹患（2015 年調査）、過去 1 年間の入院歴（2015 年調査）を有する者が多く、confounding by indication の存在が示唆された。また、肺炎球菌ワクチン接種者は、インフルエンザワクチン接種者

（2014 年、2015 年、2016 年調査）、かかりつけ医を有する者（2014 年、2015 年調査）が多く、healthy vaccinee bias の存在が示唆された。しかし、肺炎球菌ワクチン接種率の上昇に伴い、confounding by indication や healthy vaccinee bias の程度が変化している可能性がある。なお、2 回の追跡調査における死亡者は 21 人、入院・入所者は 18 人であり、肺炎球菌ワクチン接種の「死亡・入院・入所」に対する HR（95％ CI）は 0.70（0.21-2.31）であった。

別途、65 歳以上高齢者における PCV13 と PPSV23 の肺炎予防効果について、系統的レビューを行なった。該当論文 16 編についての要約は以下のとおりである。PCV13 の有効性を検討した 2 編の研究では、市中肺炎や侵襲性肺炎球菌感染症に対して有意なワクチン有効性を示していた。PPSV23 の有効性を検討した 14 編の研究では、うち 9 編が市中肺炎や肺炎球菌性肺炎、侵襲性肺炎球菌感染症、および死亡に対して有意なワクチン有効性を示していたが、結果指標によっては関連を認めなかったとする報告も散見された。日本における研究 2 編は、いずれも PPSV23 の有効性を検討した RCT であり、うち 1 編では肺炎や肺炎球菌性肺炎に対して有意なワクチン有効性を示していた。米国 ACIP は 2014 年に、65 歳以上高齢者に対しては PCV13 と PPSV23 を連続接種することを推奨した。日本でも、PCV13 と PPSV23 の連続接種の有効性に関する分析疫学研究が必要であると考えられた。

③　長谷川、森らは、札幌市の認可保育所に通う園児を対象に、急性中耳炎に対する肺炎球菌ワクチンの有効性を検討した（両向き cohort study）。札幌市内 10 ヵ所の園児 1,570 人を対象に調査依頼を行ったところ、632 人が参加した（参加率 40％）。2012 年 4 月に基礎調査を行い、既往歴やワクチン接種歴等に関する情報を収集した。また、基礎調査から 4 ヵ月毎に追跡調査を行い、ワクチン接種および急性中耳炎罹患に関する情報を得た。解析では Cox 回帰モデルを用いて、ワクチン接種の HR（95％ CI）を算出した。観察期間は、ワクチン接種者では「接種日」から「観察期間終了時（2014 年 4 月 30 日）、結果指標の発生日または満 6 歳に達するまで」とし、非接種者では「ワクチン接種可能な生後 2 ヵ月」から「観察期間終了時（2014 年 4 月 30 日）、結果指標の発生日または満 6 歳に達するまで」とした。肺炎球菌ワクチン接種者（306 人）は、非接種者（308 人）に比べて、月齢が低く、同胞数が少なく、Hib ワクチンの接種率が高かった。可能性のある交絡因子（年齢、性別、同胞数、同居喫煙者数、Hib ワクチン接種）の影響を補正したところ、肺炎球菌ワクチン接種の急性中耳炎に対する調整 HR は、対象者全体で 0.32（0.23-0.44）、3 歳未満で 0.37（0.24-0.56）、3 歳以上で 0.26（0.15-0.43）であり、肺炎球菌ワクチン接種による急性中耳炎の予防効果が示唆された。

6）新規ワクチン検討分科会

①　中野らは、岡山県の総社市（2013 年 4 月より 1 ～ 4 歳児に対する水痘ワクチンの公費助成を開始）において、水痘ワクチン接種状況、水痘患者数、水痘重症度を調査した。公費助成導入後 2 年間における対象児のワクチン接種率は 81％であった。水痘患者数は 2012 年度の 342 人から 2014 年度の 171 人に減少し、保育園の出席停止者数も有意に減少した。年齢別に検討したところ、患者数や出席停止者数は低年齢児で顕著に減少したが、年長児では明らかな減少を認めなかった。ワクチン未接種児に比べて、ワクチン 1 回接種児では、水痘の軽症化を認めた。

別途、静岡の 1 医療機関の渡航ワクチン外来を受診した 16 歳以上の者 33 人（平均 36.1 歳、男 17 人）を対象に、DTaP-sIPV の免疫原性を検討した。対象者にワクチンを 1 回皮下接種し、接種前、接種 3 週後の採血を実施した。対象者のワクチン接種歴は、母子手帳にて確認した。接種前の抗体保有割合は、ポリオ（Type Ⅰ、Ⅱ、Ⅲ：中和法）：88％、100％、76％、百日咳（抗 PT、抗 FHA：ELISA 法）：61％、76％、ジフテリア（CC 法）：97％、破傷風（KPA 法）：94％であり、接種後には破傷風 97％を除き、総て 100％の抗体保有割合を示した。接種後の有害事象は、局所反応 30％のみであり、重篤な副反応は認めなかった。

さらに、東京の 1 医療機関の渡航ワクチン外来を受診した成人 20 人（平均 37.2 歳、男 6 人）を対象に、A 型肝炎ワクチンの互換性を検討した（前向き cohort study）。対象は国産ワクチン（エイムゲン）を 2 ～ 4 週間隔で 2 回接種し、その 6 ヵ月以降に 3 回目の接種を受けるために受診した者 20 人である。3 回目接種として海外製剤の HAVRIX® を追加接種し、接種前、接種 4 週後の抗体価を CLIA 法および ELISA 法にて測定した。また、安全性の評価として、接種後 28 日間の副反応調査を行った。3

回目接種を受ける前に抗体陽性（CLIA 法で 1.0 以上、ELISA 法で 10mIU/mL と定義）を示した者は、CLIA 法で 75％、ELISA 法で 85％であったが、接種後には全例が抗体陽性を獲得した。安全性に関しては、軽微な局所反応は認めたものの重篤な副反応は認めなかった。

また、国産ワクチン（エイムゲン）を 2 回接種後に 3 年以上が経過した成人 12 人（平均 33.7 歳、男 4 人）を対象に、追加接種の効果を検討した（前向き cohort study）。2 回目接種からの経過年数は、3 年 1 人、4 年 8 人、5 年 1 人、6 年 2 人である。追加接種前の抗体陽性率は CLIA 法で 92％であり、接種後には全例で抗体陽性を獲得した。追加接種による軽微な局所反応は認めたものの重篤な副反応は認めなかった。

② 入江らは、2011 年に実施した「ポリオワクチンの互換性に関する免疫原性・安全性試験」の対象児 153 人について、接種後 5 年間の抗体持続を検討している（2013 ～ 2018 年、前向き cohort study）。なお、2011 年に実施した試験では、下記の 4 群について検討し、sOPV、wIPV、DTaP-sIPV の組み合わせ・接種順序にかかわらず、初回免疫後にはすべての者で防御レベル（NA 価 1:8）を大きく上回る抗体が誘導されたことを確認している。

・A 群（11 人）: 1 期初回（sOPV → DTaP-sIPV → DTaP-sIPV）→ 1 期追加（DTaP-sIPV）
・B 群（49 人）: 1 期初回（sOPV → wIPV → wIPV）→ 1 期追加（wIPV）
・C 群（50 人）: 1 期初回（DTaP-sIPV → DTaP-sIPV → wIPV）→ 1 期追加（wIPV）
・D 群（43 人）: 1 期初回（wIPV → wIPV → DTaP-sIPV）→ 1 期追加（DTaP-sIPV）

各群の追加免疫から 1 年後の抗体価が得られた 120 人（A 群 7、B 群 42、C 群 41、D 群 30）、2 年後の抗体価が得られた 103 人（A 群 6、B 群 36、C 群 32、D 群 29）、3 年後の抗体価が得られた 94 人（A 群 7、B 群 33、C 群 28、D 群 26）を解析対象とした。各 Sabin 株に対する幾何平均抗体価は、追加免疫後から 1 年後にかけて急速に減少した後（前年比は 0.08 ～ 0.24）、接種 2 年後以降は緩やかに減少した（前年比は 0.75 ～ 1.00）。Wild 株に対する幾何平均抗体価も同様の傾向を示したが、接種 2 年後以降の減少程度は Sabin 株よりも大きかった（前年比は 0.33 ～ 0.67）。抗体保有割合は、A 群で Wild 株 Type Ⅰに対して低下（1 年後：86％、2 年後：83％、3 年後：71％）、B 群で Sabin 株 Type Ⅲに対して低下（2 年後：97％）を認めた。他の群、他の株については 100％を維持していた。少数ではあるが、接種後 3 年間で防御レベルを下回る症例が生じたことは、我が国における潜在的リスクを反映している可能性がある。

別途、生後 2 ～ 6 ヵ月児 45 人を対象に、市販されている 2 種類の B 型肝炎ワクチン（ビームゲン：遺伝子型 C、血清型 adr、ヘプタバックスⅡ：遺伝子型 A、血清型 adw）の互換性を検討した（2015 ～ 2016 年、無作為化比較試験）。研究参加者を以下の 3 群に無作為に割付し、免疫原性・安全性を検討した。

・A 群（15 人）：ビームゲン→ヘプタバックスⅡ→ビームゲン
・B 群（15 人）：ヘプタバックスⅡ→ビームゲン→ビームゲン
・C 群（15 人）：ヘプタバックスⅡ→ヘプタバックスⅡ→ビームゲン

免疫原性は、接種前抗体陽性例 1 人を除いた 44 人を解析対象とした。幾何平均抗体価は接種毎に高くなり、3 回接種後には全例で抗体陽性（10mIU/mL 以上）となった。幾何平均抗体価および抗体陽性率のいずれにおいても、3 群間で有意差を認めなかった。安全性は、研究参加者 45 人を解析対象とした。局所反応としては発赤 7 ～ 33％、腫脹 7 ～ 13％、硬結 7 ～ 27％を認め、全身反応としては発熱 7 ～ 20％、不機嫌 7％、食欲不振 7％、下痢嘔吐胃腸炎 7％、発疹 7％を認めたが、その発現頻度は 3 群間で有意差を認めなかった。

ビームゲン、ヘプタバックスⅡのいずれの組み合わせにおいても、3 回接種後には全ての対象者が抗体陽性となり、重篤な副反応も認めなかったことから、これら 2 種類の B 型肝炎ワクチンの互換性が確認できた。

③ 原らは、佐賀県の 6 医療機関を受診した乳幼児を対象に、ロタウイルスワクチンの有効性を検討した（2011/12 ～ 2012/13 シーズン、case population study）。Case population study（スクリーニング法）によるワクチン有効性は（PPV-PCV）/｛PPV（1-PCV）｝により算出した（PPV：集団のワクチン接種割合、PCV：症例のワクチン接種割合）。集団のワクチン接種割合は、｛（対象地域へのワクチン出荷数 / 接種回数）÷対象地域の児数（≒出生数）｝× 100

（%）、により推計した。期間中に協力医療機関を受診した急性胃腸炎患者は 2011/12 シーズン：38 人、2012/13 シーズン：190 人、うち迅速検査によりロタウイルス胃腸炎と診断された患者は 2011/12 シーズン：22 人、2012/13 シーズン：87 人であった。2011/12 シーズンはロタウイルス胃腸炎患者の中にワクチン接種者がいなかったため、有効性の推計はできなかった。2012/13 シーズンの検討では、ロタウイルス胃腸炎患者のうち、ワクチン接種歴不明 7 人と 1 回目接種翌日に受診した 1 人を除外し、79 人を解析対象とした。同シーズンの PPV は 16.5%、PCV は 5.1%であり、ワクチン有効率は 72.8%と推計された。この結果は、既知の国内試験における有効率と同程度の値であり、日本におけるロタウイルスワクチンの有用性を支持している。しかし、本研究では、使用した PPV が推計値であること、二次・三次医療機関のみを対象としたこと、交絡因子の調整ができてないこと、PPV や PCV の変動で有効率が大きく変化すること、などの限界点がある。

この検討結果を受けて、2014 シーズン、佐賀市内の 6 小児科医療機関を受診した 2 ヵ月から 2 歳未満児を対象に、多施設共同症例対照研究を行い、ロタウイルスワクチンの有効性を検討した（2014 シーズン、症例対照研究）。急性胃腸炎症状で受診したすべての児に対して、ロタウイルス迅速診断検査を実施し、陽性者を症例、陰性者を対照－1（test-negative control）とした。また、症例と同時期、同年齢で同一医療機関を受診した他疾患患者を対照－2（hospital control）として選定した。これらの対象者から、自記式質問票により、ワクチン接種歴、性、年齢、出生体重、母乳保育、基礎疾患、集団保育、などの情報を得た。また、胃腸炎の臨床所見、治療状況については、病院診療録から情報を得た。症例 67 人、test-negative control 247 人を解析対象としたところ、ワクチン接種の調整 OR は 0.13（0.02-1.10）であり、有効率は 87%（−10-98%）と推計された。また、症例 67 人、hospital control 515 人を解析対象としたところ、ワクチン接種の調整 OR は 0.12（0.02-0.91）を示し、有効率は 88%（9-98%）であった。

また、2015 シーズンには、佐賀県および福岡県内の 12 小児科医療機関を受診した 2 ヵ月から 3 歳未満児を対象に、多施設共同症例対照研究（test-negative design）を行い、ロタウイルスワクチンの有効性を検討した（2015 シーズン、症例対照研究）。急性胃腸炎症状で受診したすべての児に対して、ロタウイルス迅速診断検査を実施し、陽性者を症例、陰性者を対照とした。迅速検査の結果、症例は 420 人、対照は 647 人であった。迅速診断陽性ロタウイルス胃腸炎に対するワクチン接種の調整 OR は 0.19（0.14-0.27）であり、有効率は 81%（73-86%）と推計された。

上記 2 シーズンの情報を統合し、佐賀県および福岡県内の 14 小児科医療機関を受診した 2 ヵ月から 3 歳未満児 1,412 人を対象に、ロタウイルスワクチンの有効性を検討した（2014 ～ 2015 シーズン、症例対照研究）。ロタウイルス迅速診断検査の結果、陽性者（症例）は 487 人、陰性者（対照）は 925 人であった。迅速診断陽性ロタウイルス胃腸炎に対するワクチン接種の調整 OR は 0.20（0.14-0.28）であり、有効率は 80%（72-86%）と推計された。また、点滴加療または入院を要したロタウイルス胃腸炎に対する有効率は 97%（80-92%）であり、重症例に対してより高い効果を認めた。

別途、米国予防接種諮問委員会（US-ACIP）が 2009 年に刊行したロタウイルスワクチンについての勧告「Prevention of Rotavirus Gastroenteritis Among Infants and Children: Recommendation of the Advisory Committee on Immunization Practice（ACIP）: MMWR 2009; 58: RR-2」を翻訳した。本勧告はロタウイルスワクチン接種の実施における保健医療活動の指針として学術的に参考にする価値があり、今後、我が国がロタウイルスワクチンの定期接種化を検討する際の貴重な資料になると考えられる。ACIP は、米国の乳児へのロタウイルスワクチン定期接種を勧告している。“経口生ヒト－ウシ遺伝子再集合体ロタウイルスワクチン（RotaTeq®〔RV5〕）” は生後 2 ヵ月、4 ヵ月、6 ヵ月に 3 回の経口接種、“経口弱毒生ヒトロタウイルスワクチン（Rotarix®〔RV1〕）” は生後 2 ヵ月、4 ヵ月に 2 回の経口接種としているが、特定の製剤を優先使用するような見解は示していない。その他、ロタウイルスワクチン初回接種時の最高週齢、禁忌、使用上の注意や特殊な状況について述べられている。

7）費用対効果分科会

星らは、2014 年 10 月に定期接種化された高齢者に対する肺炎球菌ワクチン接種について、助成対象

に関する費用効果分析を行った。現行の助成対象（65 歳、70 歳、75 歳、80 歳、85 歳、90 歳、95 歳、100 歳以上）を「分割ストラテジー」と定義し、「65 ～ 80 歳年齢限定一括接種ストラテジー」、「65 歳以上一括接種ストラテジー」と比較した。侵襲性肺炎球菌感染症（IPD）の罹患率、後遺症の発現率、各種費用データを用いて、マルコフ・モデルを作成した。疫学データは国内の文献から、ワクチン効果は海外の文献から引用した。その結果、「プログラムなし」と比較した場合の各ストラテジーの 1QALY 獲得あたりの増分費用は、「分割ストラテジー」29,107,379 円、「65 ～ 80 歳年齢限定一括接種ストラテジー」11,232,203 円、「65 歳以上一括接種ストラテジー」13,038,976 円であった。高齢者に対する 23 価肺炎球菌ワクチンは、ストラテジーに拘らず、cost-effective とは言い難いが、「65 ～ 80 歳年齢限定一括接種ストラテジー」または「65 歳以上一括接種ストラテジー」の方が、現行の「分割ストラテジー」よりも費用効果に優れることが示唆された。

別途、高齢者に対する肺炎球菌ワクチン接種について、現行の助成対象（65 歳、70 歳、75 歳、80 歳、85 歳、90 歳、95 歳、100 歳以上）とした「現行 PPSV23 接種ストラテジー」と、今後 PCV13 が定期接種に加わった場合の「PPSV23・PCV13 選択可能接種ストラテジー」を設定し、費用効果分析を行った。「PPSV23・PCV13 選択可能接種ストラテジー」では、被接種者のうち PCV13 を選択する割合を 10％～ 90％の 10％間隔で合計 8 レベル設定した。侵襲性肺炎球菌感染症（IPD）および非侵襲性疾患の罹患率、後遺症の発現率、各種費用データを用いて、マルコフ・モデルを作成した。疫学データは国内の文献から、ワクチン効果は海外の文献から引用した。その結果、「現行 PPSV23 接種ストラテジー」と比べて「PPSV23・PCV13 選択可能接種ストラテジー」では、PCV13 の使用割合に拘らず、QALY の獲得と罹病のための医療費の減少を認めた。「現行 PPSV23 接種ストラテジー」と比較した「PPSV23・PCV13 選択可能接種ストラテジー」の 1QALY 獲得あたりの増分費用は約 37.9 万円であり、定期接種助成対象ワクチンとして PCV13 の導入は費用対効果に優れていると考えられた。

さらに、2016 年 3 月、国産乾燥弱毒生水痘ワクチンの効能・効果に「50 歳以上の者に対する帯状疱疹の予防」が追加承認されたことを受けて、高齢者に対する帯状疱疹ワクチン接種についての費用効果分析を行なった。接種年齢の異なる 4 つの接種ストラテジー（① 65 ～ 84 歳、② 70 ～ 84 歳、③ 75 ～ 84 歳、④ 80 ～ 84 歳）を設定し、「接種プログラムなし」と比較した。マルコフ・モデルには、発症なし、帯状疱疹発症後回復する、神経痛に発展後回復する、死亡の 4 つのヘルス・ステータスを設定し、生存対象者が 100 歳になるまで回した。1 回接種当たりの費用は 10,000 円と仮定し、モデルに組み入れる疫学データは国内の文献から、ワクチン効果は海外の文献から、それぞれ引用した。1QALY 獲得あたりの増分費用は 280 万円（65 ～ 84 歳）から 360 万円（80 ～ 84 歳）であった。65 歳以上高齢者に対する帯状疱疹予防接種は費用対効果に優れ、定期予防接種に含める候補として検討する価値があることが示唆された。

8）微生物検索・病原診断分科会

① 前田、菅野、加瀬らは、児童養護施設入所者 30 人（年齢 7 ～ 19 歳）を対象に、流行野生株（A 型）に対する抗体誘導を検討した（2013/14 シーズン、前向き cohort study）。抗体価測定のための採血は、接種前および接種 1 ヵ月後に行い、ワクチン株と 2013/14 シーズンの流行野生株（A/Osaka/52/2014（H1pdm）、A/Osaka/49/2014（H3））に対する HI 価を測定した。H1pdm に関して、ワクチン株 A/California/7/2009 に対する GMT は 122（接種前）→ 403（接種後）に上昇し（MFR：3.3）、sR は 10％、sP は 83 → 97％に上昇した。流行野生株 A/Osaka/52/2014 に対しても、GMT は 134 → 221 に上昇し（MFR：1.6）、sR：10％、sP：77 → 93％に上昇した。一方、H3 に関しては、ワクチン株 A/Texas/50/2012 に対する GMT は 122 → 200 に上昇し（MFR：1.6）、sR：10％、sP：93 → 100％を示したものの、流行野生株 A/Osaka/49/2014 に対しては GMT は 20 → 27（MFR：1.4）、sR：7％、sP：30 → 50％であり、十分な抗体レベルには到らなかった。

同内容の調査を 2014/15 シーズンも継続して実施した（2014/15 シーズン、前向き cohort study）。盛岡市の児童養護施設入所者 27 人（年齢 9 ～ 19 歳）を対象として、ワクチン株と流行野生株（A/Osaka/18/2015（H3）、A/Osaka/16/2015（H3））に対する HI 価を評価した。ワクチン

株 A/NewYork/39/2015(H3) に対する GMT は 245 → 403 に上昇し（MFR：1.6)、sR は 19 %、sP は 100 %→ 100 %を示した。一方、流行野生株 A/Osaka/18/2015(H3) に対する GMT は 16 → 30（MFR：1.8)、sR：15 %、sP：26 → 48 %であった。また、A/Osaka/16/2015(H3) に対しては、GMT は 18 → 27(MFR：1.5)、sR：15 %、sP：26 → 52 %であり、流行野生株に対する免疫原性は汎用されている EMA 基準には達しなかった。通常、インフルエンザワクチンの抗体誘導能はワクチン株に対する抗体価によって評価されている。しかし、ワクチンの臨床効果を念頭に置いて抗体誘導能を議論する場合は、流行野生株に対する抗体価を参考にすることが重要であり、今後も知見を継続して蓄積していく必要がある。

別途、2010/11 ～ 2013/14 シーズンにインフルエンザワクチン接種を受けた児童養護施設入所者を対象に、B 型（ビクトリア系統、山形系統）に対する抗体応答を検討した（2010/11 ～ 2013/14 シーズン、前向き cohort study)。各シーズンの対象者は、2010/11 シーズン 28 人、2011/12 シーズン 30 人、2012/13 シーズン 33 人、2013/14 シーズン 30 人である。なお、2010/11 と 2011/12 シーズンのワクチンはビクトリア系統を含有しており、2012/13 と 2013/14 シーズンのワクチンは山形系統を含有している。接種前および接種 1 ヵ月後の血清について、ビクトリア系統と山形系統の HI 価を測定した。ビクトリア系統含有ワクチンを接種した前 2 シーズンでは、ビクトリア系統に対する sP は 39%→ 46%（2010/11 シーズン)、33 %→ 85 %（2011/12 シーズン）に上昇し、山形系統に対しても sP：39 %→ 68%（2010/11 シーズン)、33%→ 47%（2011/12 シーズン）に上昇した。一方、山形系統含有ワクチンを接種した後 2 シーズンでは、山形系統に対する sP は 8%→ 27%（2012/13 シーズン)、43%→ 60%（2013/14 シーズン）に上昇したが、ビクトリア系統に対しては sP：15%→ 21%（2012/13 シーズン)、60%→ 63%（2013/14 シーズン）と軽度の増加を認めるにとどまった。GMT についても同様の傾向を示し、山形系統に対して 13 → 25(2012/13 シーズン)、24 → 31(2013/14 シーズン）に軽度上昇したものの（MFR は、それぞれ 1.9 と 1.2)、ビクトリア系統に対しては 20 → 22(2012/13 シーズン)、31 → 31(2013/14 シーズン）と、ほとんど変化を認めなかった（MFR はそれぞれ 1.1 と 1.0)。過去 4 シーズンを通じて、B 型に対する免疫応答は、非常に低いと考えられた。交差免疫については、ビクトリア系統含有ワクチンを接種したシーズンでは山形系統への免疫誘導が示唆されたが、山形系統含有ワクチンを接種したシーズンではビクトリア系統への免疫誘導は明らかではなかった。

② 森川らは、大阪府におけるインフルエンザ流行のウイルス学的特徴を検討した（2014/15 ～ 2015/16 シーズン)。2014/15 シーズンは例年よりも早く流行が始まり年末にピークを迎えたが、流行規模は中規模であった。主流行株は AH3 亜型ウイルスで、その抗原性は前シーズンと比べて大きく異なっていた。2015/16 シーズンは 2014/15 シーズンよりも規模が大きかったが、流行曲線は遅延型であった。主流行株は AH1pdm09 亜型ウイルスと B 型ウイルス（山形系統）であった。それらの抗原性は 2014/15 シーズンと類似していた。連続した 2 シーズンを比較すると、流行規模、流行時期、流行期間に差異を認めた。また、主流行株は全く異なっており、インフルエンザの疫学研究が画一的にできないことを示していると考えられた。

9）広報啓発分科会

小笹、入江、福島、大藤、伊藤を中心に、平成 26 年度 21 人、平成 27 年度 20 人、平成 28 年度 21 人の班員が共同して、米国疾病管理センター（CDC）の予防接種諮問委員会（US-ACIP）の勧告 2014 年版「Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practice（ACIP）— United States, 2014–2015 Influenza Season(MMWR 2014; 63（32): 691-697)」、2015 年版「Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practice（ACIP）— United States, 2015–2016 Influenza Season（MMWR 2015; 64（30): 818-825)」、および 2016 年版「Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practice（ACIP）— United States, 2016–2017 Influenza Season(MMWR 2016; 65（5): 1-52)」を翻訳し、一般財団法人・日本公衆衛生協会より出版した（「インフルエンザの予防と対策、2014 年度版」小笹晃太郎・入江伸・福島若葉・大

藤さとこ（編集）、廣田良夫・葛西健（監修);「インフルエンザの予防と対策、2015 年度版」小笹晃太郎・入江伸・福島若葉・大藤さとこ（編集）、廣田良夫・葛西健（監修);「インフルエンザの予防と対策、2016 年度版」小笹晃太郎・入江伸・福島若葉・大藤さとこ・伊藤一弥（編集）、廣田良夫・葛西健（監修））。本勧告はインフルエンザの予防と対策において世界標準に位置づけられている。インフルエンザに関する最新の知識を普及させるために広く活用されるものと考える。

F. 健康危険情報

不活化ポリオワクチン接種後、抗体価の減衰が速い者がいる。特に、初回・追加の計 4 回、Sabin 株由来ワクチンのみを接種された者で、Wild 株に対する抗体価が減衰しやすい傾向が示唆された。

【書　籍】

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
小笹晃太郎、入江　伸、福島若葉、大藤さとこ、鈴木幹三(アドバイザー)	インフルエンザの予防と対策	廣田良夫、葛西 健 (監)	米国疾病管理センター(CDC)予防接種諮問委員会 (ACIP) 勧告、インフルエンザの予防と対策	(財)日本公衆衛生協会	東京	2016	
岡田賢司	長引く咳	楠原浩一	専門医がリードする小児感染症ケースカンファレンス 2016	南山堂	東京	2016	143-8
岡田賢司	DTP-IPV ワクチン①百日咳ワクチンを中心に	渡辺 博	小児の予防接種ハンドブック	総合医学社	東京	2016	108-15
中野貴司	DPT-IPV ワクチン③不活化ポリオワクチンを中心に	渡辺 博	小児の予防接種ハンドブック	総合医学社	東京	2016	124-35
岡田賢司	成人・高齢者から小児への感染症	福永慶隆	小児科	金原出版	東京	2016	709-16
岡田賢司	百日咳の臨床診断	山田俊幸	臨床検査第 60 巻第 7 号	医学書院	東京	2016	762-6
中野貴司	第 8 章. インフルエンザワクチンの有効性、副反応	菅谷憲夫	インフルエンザ診療ガイド 2016-17	日本医事新報社	東京	2016	157-66
小笹晃太郎、入江 伸、福島若葉、大藤さとこ、他	インフルエンザの予防と対策	廣田良夫、葛西 健	米国疾病管理センター(CDC)予防接種諮問委員会 (ACIP) 勧告、インフルエンザの予防と対策	(財)日本公衆衛生協会	東京	2015	
鈴木幹三、近藤亨子、廣田良夫	成人の肺炎球菌ワクチンと、インフルエンザワクチンの同時接種の安全性と効果について	編著：中野貴司	予防接種の現場で困らない「まるわかりワクチンQ&A」	日本医事新報社	東京	2015	239-40
鈴木幹三、足立　暁、菅　栄	成人の肺炎球菌ワクチンを複数回接種すると局所の副反応が出やすいと聞きますが、再接種の安全性について	編著：中野貴司	予防接種の現場で困らない「まるわかりワクチンQ&A」	日本医事新報社	東京	2015	241-43
福島若葉	日本の不活化インフルエンザワクチンの有効性について、対象年齢別、評価指標別（発病予防・死亡回避など）に	編著：中野貴司	予防接種の現場で困らない「まるわかりワクチンQ&A」	日本医事新報社	東京	2015	307-8
福島若葉	2009 年に出現した A(H1N1) pdm09 ウイルスのワクチンによる予防効果について	編著：中野貴司	予防接種の現場で困らない「まるわかりワクチンQ&A」	日本医事新報社	東京	2015	309-10
中野貴司	ポリオワクチンを成人に接種する場合はありますか。昭和 50 ～ 52 年生まれの世代はポリオに対する免疫が低いと聞いたのですが、追加接種をしたほうがよいですか。	編著：中野貴司	予防接種の現場で困らない「まるわかりワクチンQ&A」	日本医事新報社	東京	2015	138-40

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
中野貴司	海外へ行く児の予防接種	編著： 庵原俊昭、 寺田喜平	よくわかる予防接種のキホン	中外医学社	東京	2015	94 -105
岡田賢司	百日咳ワクチンの効果 / 問題点と対策	広報委員会	日本小児科医会会報 49	日本小児科医会	東京	2015	31-5
岡田賢司	百日咳 : 大人からうつる	石和田稔彦他	小児科診療 78(7)	診断と治療社	東京	2015	883-89
岡田賢司	百日咳	福井次也他	今日の治療指針 2015	医学書院	東京	2015	237-38
岡田賢司	百日咳	市川光太郎	小児救急治療ガイドライン改訂第 3 版	診断と治療社	東京	2015	170-4
岡田賢司	百日咳、パラ百日咳	水口　雅他	今日の小児治療指針第 16 版	医学書院	東京	2015	361-2
小笹晃太郎、入江　伸、福島若葉、大藤さとこ、他	インフルエンザの予防と対策	廣田良夫、葛西　健	米国疾病管理センター (CDC) 予防接種諮問委員会 (ACIP) 勧告、インフルエンザの予防と対策	(財) 日本公衆衛生協会	東京	2014	
鈴木幹三	感染症へのアプローチ : 感染症の予防、感染症の分類、感染症新法	井村裕夫	わかりやすい内科学 第4版	文光堂	東京	2014	385-9
中野貴司	予防接種各論、5 章. ポリオワクチン	五十嵐隆	小児科臨床ピクシス 4 ; 予防接種　全訂新版	中山書店	東京	2014	136-45
中野貴司	インフルエンザワクチンの有効性、副反応	菅谷憲夫	インフルエンザ診療ガイド 2014-2015	日本医事新報社	東京	2014	134-42

【雑　誌】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hara M, Hirota Y	Basic principle of population-based cohort study to evaluate influenza vaccine effectiveness among elderly Japanese.	Vaccine			in press
Ohfuji S, Deguchi M, Tachibana D, Koyama M, Takagi T, Yoshioka T, Urae A, Fukushima W, Hirota Y for the Osaka Pregnant Women Influenza Study Group.	Estimating influenza disease burden among pregnant women: application of self-control method.	Vaccine			2017 in press
Ohfuji S, Okada K, Nakano T, Ito H, Hara M, Kuroki H, Hirota Y.	Control selection and confounding factors: A lesson from a Japanese case-control study to examine acellular pertussis vaccine effectiveness	Vaccine			2016 in press
Hoshi SL, Kondo M, Okubo I.	Economic evaluation of routine infant rotavirus immunisation programme in Japan.	Hum Vaccin Immunother.	Oct 20:0.		2016 in press
Hasegawa J, Mori M, Ohnishi H, Tsugawa T, Hori T, Yoto Y, Tsutsumi H	Pneumococcal vaccination reduces the risk of community-accuired pneumonia in children.	Pediatr Int.			2016 in press
Washio M, Kondo K, Fujisawa N, Harada E, Tashiro H, Mizokami T, Nogami H, Iwanaga T, Nakanishi Y, Suzuki K, Ohfuji S, Fukushima W, Hirota Y; Kyushu Task Force for CAP Risk in the Elderly.	Hypoalbuminemia, influenza vaccination and other factors related to the development of pneumonia acquired outside hospitals in southern Japan: A case-control study.	Geriatr Gerontol Int.	16	223-9	2016
Nakashima K, Aoshima M, Ohfuji S, Suzuki K, Katsurada M, Katsurada N, Misawa M, Otsuka Y, Kondo K & Hirota Y	Immunogenicity of trivalent influenza vaccine in patients with lung cancer undergoing anticancer chemotherapy.	Hum Vaccin Immunother.	7	1-8	2016
Araki K, Hara M, Sakanishi Y, Shimanoe C, Nishida Y, Matsuo M, Tanaka K.	Estimating rotavirus vaccine effectiveness in Japan using a screening method.	Hum Vaccin Immunother.	12(5)	1244-9	2016
Hara M, Hanaoka T, Maeda K, Kase T, Ohfuji S, Fukushima W, Hirota Y	Immunogenicity and efficacy of A/H1N1pdm vaccine among subjects with severe motor and intellectual disability in the 2010/11 influenza season.	J Epidemiol	26(6)	300-6	2016
Asada K, Kamiya H, Suga S, Nagao M, Ichimi R, Fujisawa T, Umemoto M, Tanaka T, Ito H, Tanaka S, Ido M, Taniguchi K, Ihara T, Nakano T	Rotavirus vaccine and health-care utilization for rotavirus gastroenteritis in Tsu City, Japan	WPSAR (Western Pacific Surveillance and Response Journal)	7(4)	1-9	2016
Hara M, Fukuoka M, Tashiro K, Ozaki I, Ohfuji S, Okada K, Nakano T, Fukushima W, Hirota Y.	Pertussis outbreak in university students and evaluation of acellular pertussis vaccine effectiveness in Japan.	BMC Infect Dis	15	45	2015

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakata K, Kashiwagi M, Masuda M, Shigehara S, Oba C, Murata S, Kase T, Komano JA.	A child with acute encephalopathy associated with quadruple viral infection.	Front Pediatr.	3(26)	1-4	2015
Nakata K, Fujieda M, Miki H, Fukushima W, Ohfuji S, Maeda A, Kase T, Hirota Y.	Detection of influenza vaccine effectiveness among nursery school children: lesson from a season with cocirculating respiratory syncytial virus.	Hum Vaccin Immunother.	11(3)	545-52	2015
Kondo K, Suzuki K, Washio M, Ohfuji S, Fukushima W, Maeda A, Hirota Y and the Pneumonia in Elderly People Study Group:	Association between monovalent influenza A (H1N1) pdm09 vaccine and pneumonia among the elderly in the 2009-2010 season in Japan: A case-control study.	Hum Vaccin Immunother.	11(5)	1088-93	2015
Saito T, Ohfuji S, Matsumura T, Saito T, Maeda K, Maeda A, Fukushima W, Fujimura H, Sakoda S, Hirota Y.	Safety of a Pandemic Influenza Vaccine and the Immune Response in Patients with Duchenne Muscular Dystrophy.	Intern Med.	54(10)	1199-205	2015
Morikawa S, Kohdera U, Hosaka T, Ishii K, Akagawa S, Hiroi S, Kase T.	Seasonal variations of respiratory viruses and etiology of human rhinovirus infection in children.	J Clin Virol.	73	14-9	2015
Morikawa S, Hiroi S, Kase T.	Detection of respiratory viruses in gargle specimens of healthy children.	J Clin Virol.	64	59-63	2015
Matsumoto H, Ohfuji S, Watanabe K, Yamagami H, Fukushima W, Maeda K, Kamata N, Sogawa M, Shiba M, Tanigawa T, Tominaga K, Watanabe T, Fujiwara Y, Hirota Y, Arakawa T.	Booster influenza vaccination does not improve immune response in adult inflammatory bowel disease patients treated with immunosuppressives: a randomized controlled trial.	J Gastroenterol	50(8)	876-86	2015
Hasegawa J, Mori M, Showa S, Matsushima A, Ohnishi H, Tsugawa T, Yoto Y, Tsutsumi H	Pneumococcal vaccination reduced the risk of acute otitis media:Cohort study	Pediatr Int.	57	582-5	2015
Hoshi SL, Kondo M, Okubo I.	Economic Evaluation of Immunisation Programme of 23-Valent Pneumococcal Polysaccharide Vaccine and the Inclusion of 13-Valent Pneumococcal Conjugate Vaccine in the List for Single-Dose Subsidy to the Elderly in Japan.	PloS One	10(10)	:e0139140	2015
Ohfuji S, Okada K, Nakano T, Ito H, Hara M, Kuroki H, Hirota Y.	Effectiveness of acellular pertussis vaccine in a routine immunization program: A multicenter, case-control study in Japan.	Vaccine	33(8)	1027-32	2015

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Okada C, Fujieda M, Fukushima W, Ohfuji S, Kondo K, Maeda A, Nakano T, Kaji M, Hirota Y, for the Influenza Vaccine Epidemiology Study Group.	Reactogenicity of trivalent inactivated influenza vaccine in young children: Pronounced reactions by previous successive vaccinations.	Vaccine	33(30)	3586-91	2015
Mugitani A, Ito K, Irie S, Eto T, Ishibashi M, Ohfuji S, Fukushima W, Maeda A, Hirota Y, for the Fukuoka Pediatricians Group for Vaccine Efficacy.	Immunogenicity of the trivalent inactivated influenza vaccine in young children less than 4years of age, with a focus on age and baseline antibodies.	Clin Vaccine Immunol.	21(9)	1253-60	2014
Egawa Y, Ohfuji S, Fukushima W, Yamazaki Y, Morioka T, Emoto M, Maeda K, Inaba M, Hirota Y.	Immunogenicity of influenza A(H1N1)pdm09 vaccine in patients with diabetes mellitus: with special reference to age, body mass index, and HbA1c.	Hum Vaccin Immunother.	10(5)	1187-94	2014
Ide Y, Imamura Y,Ohfuji S, Fukushima W, Ide S, Tsutsumi C, Koga M, Maeda K, Hirota Y.	Immunogenicity of a monovalent influenza A(H1N1)pdm09 vaccine in patients with hematological malignancies.	Hum Vaccin Immunother.	10(8)	2387-94	2014
Hagihara Y, Ohfuji S, Watanabe K, Yamagami H, Fukushima W, Maeda K, Kamata N, Sogawa M, Shiba M, Tanigawa T, Tominaga K, Watanabe T, Fujiwara Y, Hirota Y, Arakawa T.	Infliximab and/or immunomodulators inhibit immune responses to trivalent influenza vaccination in adults with inflammatory bowel disease.	J Crohns Colitis	8(3)	223-33	2014
Ohfuji S, Fukushima W, Sasaki Y, Tamori A, Kurai O, Kioka K, Maeda K, Maeda A, Hirota Y.	Influenza A(H1N1)pdm09 vaccine effectiveness and other characteristics associated with hospitalization in chronic liver disease patients.	Liver Int.	34(5)	700-6	2014
Mori M, Hasegawa J, Showa S, Matsushima A, Ohnishi H, Yoto Y, Tsutsumi H.	Effectiveness of influenza vaccine in children in day-care centers of Sapporo.	Pediatr Int.	56	53-6	2014
Hoshi SL, Kondo M, Okubo I.	Economic evaluation of vaccination programme of mumps vaccine to the birth cohort in Japan.	Vaccine	32(33)	4189-97	2014
加瀬哲男	身近な感染症も海外から入ってきます	日本旅行医学会学会誌			印刷中
福島若葉	6 歳未満児におけるインフルエンザワクチンの有効性：2013/14 および 2014/15 シーズン（厚生労働省班研究報告として）	病原微生物検出情報（IASR）	37(11)	230-1	2016
加瀬哲男	ほかの地域はどうしてる？施設のラウンドに至るまで（特集 加算 (1)(2) 施設必読！講義 30 分 + ラウンド 30 分 療養型病院と高齢者施設の感染対策）	INFECTION CONTROL	25(10)	931-7	2016
岡田賢司	百日咳	医学と薬学	73(2)	149-55	2016
福島若葉	インフルエンザワクチン：現状と課題	医薬ジャーナル	52(10)	107-11	2016

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
山口優子、諸熊一則、目野郁子、岡田賢司、宮﨑千明、植田浩司	北九州地方における看護学生（1994 ～ 2011 年入学）を対象とした百日咳、ジフテリア、破傷風の血清疫学調査	感染症学会雑誌	90(4)	473-9	2016
中野貴司	海外旅行・海外長期滞在と感染症	小児科	57(6)	724-31	2016
中野貴司	髄膜炎菌ワクチン - 日本での適応	小児科診療	79(4)	529-34	2016
加瀬哲男、森川佐依子	原因ウイルスとウイルス別の伝播方法（特集 上気道感染症を科学する）	日本胸部臨床	75(9)	946-56	2016
山本裕子、三宅真砂子、上田美子、高杉尚志、中野貴司	水痘ワクチン公費助成、定期接種化の効果	日本小児科学会雑誌	120(6)	978-85	2016
中野貴司	トラベラーズワクチンの現状と問題点	日本内科学会雑誌	105(11)	2146-53	2016
脇本寛子、矢野久子、鈴木幹三、他：	外来職員における麻疹、風疹、流行性耳下腺炎、水痘に対する免疫獲得状況ー「院内保管記録」と「今回の血清抗体価測定結果・質問紙調査結果」との比較によるワクチン接種プログラム構築に向けた検討ー	名古屋市立大学看護学部紀要	15	7-14	2016
中野貴司	ワクチン接種スケジュールと同時接種	薬局	67(3)	352-61	2016
福島若葉、廣田良夫	「ワクチン、特にインフルエンザワクチンは効かないの?」～「疫学」の視点から答えるために～	薬局	67(3)	389-94	2016
中野貴司	紛争とポリオ	臨床とウイルス	44(1)	11-7	2016
鷲尾昌一、近藤亨子、鈴木幹三、他	肺炎球菌ワクチン接種高齢者と非接種高齢者の特徴ー福岡県における高齢者肺炎の症例対照研究参加者の解析結果よりー	臨牀と研究	93(8)	1109-12	2016
加瀬哲男	ワクチンと Vaccine Preventable Diseases(VPD : によって予防可能な疾病)- 最近の話題 -	Makoto	172	2-7	2015
福島若葉、廣田良夫	疫学によるインフルエンザワクチンの有効性評価	Pharma Medica.	33(11)	47-51	2015
鈴木幹三、中村　敦、太田千晴、他	高齢者の呼吸器感染症予防―インフルエンザワクチン・肺炎球菌ワクチンの併用接種向上への取り組みとその効果	感染と抗菌薬	18	271-8	2015
中野貴司	侵襲性髄膜炎菌感染症と予防ワクチン	小児科臨床	68(10)	1963-71	2015
中野貴司	A 型肝炎ワクチン	小児科臨床	68(12)	2625-30	2015
中野貴司	15 歳以上の 3 種混合 (DPT) ワクチン未接種者にどう対応する？	日本医事新報	No.4776	66-7	2015
岡田賢司、田中正章、宗　稔	小児科医療従事者における百日咳血清疫学の前方視・縦断研究	日本小児科学会雑誌	119(11)	1643-50	2015
脇本寛子、矢野久子、鈴木幹三、他	外来職員における麻疹、風疹、流行性耳下腺炎、水痘に対する免疫獲得状況とその解析	名古屋市立大学看護学部紀要	14	35-42	2015
矢野久子、脇本寛子、鈴木幹三、他	外来職員のB型肝炎に対する免疫獲得状況と課題～医療職と非医療職の比較～	名古屋市立大学看護学部紀要	14	43-50	2015

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
岡田賢司	長引く咳：百日咳	臨床とウイルス	43(1)	5-12	2015
原めぐみ	DTaP ワクチンの成人への接種	臨床とウイルス	43(1)	47-52	2015
福島若葉	Vaccine Epidemiology: Principles and Methods -- 症例・対照研究（case-control study）によるインフルエンザワクチンの有効性評価	臨床医薬	31(4)	309-16	2015
鈴木幹三	Vaccine Epidemiology:Principles and Methods 高齢者肺炎の症例対照研究	臨床医薬	31(4)	329-34	2015
福島若葉	Vaccine Epidemiology: Principles and Methods -- Self-controlled case series study: オセルタミビル服薬と異常行動・異常言動の関連に対するデザイン適用の試み	臨床医薬	31(4)	339-42	2015
大藤さとこ	Vaccine Epidemiology: Principles and methods 妊婦における季節性インフルエンザの健康影響に関する調査　－ Self-control method －	臨床医薬	31(4)	317-23	2015
岡田賢司	Vaccine Epidemiology:Principles and Methods 百日咳ワクチンの有効性に関する症例対照研究	臨床医薬	31(4)	324-28	2015
原めぐみ	Vaccine Epidemiology:Principles and Methods コーホート研究による地域在住高齢者におけるインフルエンザワクチンの有効性評価	臨床医薬	31(4)	300-8	2015
大藤さとこ	Vaccine Epidemiology: Principles and methods ワクチン接種の有害事象　Self-control method による因果性検証（事例紹介）	臨床医薬	31(4)	335-38	2015
福島若葉、廣田良夫	インフルエンザワクチンの有効性	Medical Science Digest	40(10)	485-7	2014
中野貴司	新しく導入されたワクチン～不活化ポリオワクチンを含むワクチン	チャイルドヘルス	17(9)	629-33	2014
中野貴司	ポリオワクチン	感染症内科	2(3)	264-73	2014
鈴木幹三、青山恵美、住田千鶴子、矢野久子	市中におけるノロウイルス感染制御、高齢者介護施設および在宅介護の場合	感染対策 ICT ジャーナル	9(4)	328-34	2014
鈴木幹三、田中世津子、高野英雄、青木　誠	長期療養型施設とノロウイルス	在宅ケアの感染対策と消毒	12(1)	2-3	2014
中野貴司	インフルエンザワクチン	小児科臨床	67(4)	569-74	2014
大藤さとこ、廣田良夫	【日常診療とワクチン】糖尿病患者とワクチン	成人病と生活習慣病	44(12)	1436-9	2014
坂西雄太、原めぐみ、福森則男、草場鉄周、田中恵太郎、杉岡隆	わが国のプライマリ・ケア医の定期接種及び任意接種ワクチンの接種状況、接種推奨割合および接種推奨の障壁	日本プライマリ・ケア連合学会誌	37	254-9	2014
中野貴司	ワクチンの定期接種化	日本医事新報	4720	25-30	2014

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
矢野久子、長崎由紀子、脇本寛子、堀田法子、鈴木幹三	外来における感染症患者の早期発見のための症状・徴候による問診票の評価	名古屋市立大学看護学部紀要	13	37-42	2014

RESEARCH PAPER

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Immunogenicity of influenza A(H1N1)pdm09 vaccine in patients with diabetes mellitus

With special reference to age, body mass index, and HbA1c

Yumi Egawa[1,*], Satoko Ohfuji[1], Wakaba Fukushima[1], Yuko Yamazaki[2], Tomoaki Morioka[2], Masanori Emoto[2], Kazuhiro Maeda[3], Masaaki Inaba[2], and Yoshio Hirota[1]

[1]Department of Public Health; Faculty of Medicine; Osaka City University; Osaka, Japan; [2]Department of Metabolism Endocrinology, and Molecular Medicine; Faculty of Medicine; Osaka City University; Osaka, Japan; [3]Kannonji Institute; Research Foundation for Microbial Diseases of Osaka University; Kagawa, Japan

Keywords: diabetes mellitus; influenza A(H1N1)pdm09 vaccine, predictors, immunogenicity, HbA1c, BMI, age

Abbreviations: DM, diabetes mellitus; HbA1c, hemoglobin A1c; ACIP, Advisory Committee on Immunization; WHO, World Health Organization; HAI, hemagglutination-inhibition; GMT, geometric mean titer; GMTR, geometric mean titer ratio; OR, odds ratio; CI, confidence interval

Subjects with diabetes mellitus are considered to be at high risk of influenza infection and influenza-associated complications. To evaluate the immunogenicity of the influenza A(H1N1)pdm09 vaccine among these subjects, we performed a prospective cohort study and measured hemagglutination inhibition antibody titers at baseline and 3 weeks after vaccination in 49 patients. No serious adverse events were reported. We were able to perform analyses for 48 patients, after excluding one patient with suspected infection. The vaccine induced a rise of about 9-fold in the mean antibody level. The sero-response proportion was 79%, and the sero-protection proportion was 73%. Patients with older age and lower body mass index tended to show lower immune response. Multivariate analysis indicated an independent negative effect of hemoglobin A1c level on the sero-protection proportion. A single A(H1N1)pdm09 vaccination achieved a sufficient level of immunity among diabetic patients, but both clinicians and patients should be aware of the potential for reductions in immune response.

Introduction

Patients with diabetes mellitus (DM) are presumed to have abnormalities in immune function and are classified as a high-risk group for developing complications, hospitalizations and death related to influenza.[1-3] According to the Advisory Committee on Immunization Practices (ACIP) in the United States, vaccinating high-risk individuals before influenza season each year is the most effective measure for reducing the impact of influenza.[4] Annual influenza vaccination has therefore long been recommended for these individuals.[1,5]

In June 2009, the World Health Organization (WHO) declared a global pandemic of the influenza A(H1N1)pdm09 and identified chronic medical conditions as being specific risks for infection.[6] As a result, many diabetic patients received H1N1 vaccination according to the recommendations of the WHO.[7] However, these recommendations were based on clinical trials in healthy individuals,[8] and little is known about the immunogenicity of the vaccine in high-risk groups, including diabetic patients. The present study investigated immunogenicity of the vaccine in diabetic individuals and tried to identify factors affecting immune response.

Results

Study subjects

We excluded 1 patient in whom both pre- and post-vaccination titers were 1:160, as subclinical infection was suspected in that patient. Among the total of 48 diabetic patients, 7 patients with type 1 DM (3 men, 4 women; mean age (± standard deviation), 47.3 ± 14.6 y) and 41 patients with type 2 DM (33 men, 8 women; mean age, 59.8 ± 11.4 y) were analyzed. Subject characteristics are shown in **Table 1**. Mean hemoglobin (Hb)A1c level was 7.44%, and more than half of the patients were treated with insulin. No patients were receiving steroid therapy or undergoing dialysis. Type 1 patients were younger and the proportion of males was lower compared with type 2 patients. The distribution of HbA1c levels and body mass index (BMI) did not differ between groups.

Immune response

Results of antibody response by background factors are summarized in **Table 2**. The vaccine induced a mean increase in hemagglutination-inhibition (HAI) antibody level of 9-fold ($P < 0.0001$). Sero-response proportion was 79% (95% CI, 62–97%), and the sero-protection proportion was 73% (95% CI, 60–86%). The corresponding sero-conversion proportion

*Correspondence to: Yumi Egawa; Email: yegawa@mbr.nifty.com
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Table 1. Characteristics of study participants

Characteristics	Study subjects				
	n (%)	Total (N=48)	Type 1 (N=7)	Type 2 (N=41)	*P* value
Sex	male	36 (75)	3 (43)	33 (81)	0.043
Age (y)	mean (S.D.)	57.9 (12.5)	47.3 (14.6)	59.8 (11.4)	0.029
	median (range)	61.0 (26–75)	45.0 (26–66)	62.0 (28–75)	
Duration of disease(y)	mean (S.D.)	7.0 (15.0)	8.0 (8.2)	12.9 (10.0)	0.235
	median (range)	9.5 (1–37)	5.0 (3–26)	10.0 (1–37)	
HbA1c	mean (S.D.)	7.44 (1.45)	7.81 (1.06)	7.37 (1.51)	0.160
	median (range)	7.30 (4.9–14.4)	8.20 (5.9–9.0)	7.30 (4.9–14.4)	
Body mass index (kg/m^2)	mean (S.D.)	23.8 (3.3)	23.0 (2.1)	23.9 (3.5)	0.365
	median (range)	23.5 (17.0–32.2)	22.2 (20.8–27.3)	23.5 (17.0–32.2)	
Treatment of DM	no medication	5 (10)	0 (0)	5 (12)	
	internal use only	16 (33)	0 (0)	16 (39)	
	insulin +	27 (56)	7 (100)	20 (49)	
Treatment of hypertension	received	21 (44)	1 (14)	20 (49)	0.096
Treatment of hyperlipidemia	received	17 (35)	2 (29)	15 (37)	0.698
Steroid therapy	received	0	0	0	
Dialysis	received	0	0	0	

was 73% (95% confidence interval [95% CI], 60–86%). Older patients showed a smaller immune response, as reflected in post-vaccination geometric mean titer (GMT) (P = 0.027) and sero-protection proportion (P = 0.059). Lower BMI was associated with lower sero-response proportion, displaying a clear dose-response relationship (P = 0.006). This relationship remained unchanged (trend P = 0.008) even after considering the effects of potential confounders (**Table 3**). The odds ratio (OR) for sero-response among those subjects with highest HbA1c (≥7.6%) was low, although no significant relationship was apparent.

Predictors of immune response in terms of sero-protection proportion were also analyzed (**Table 4**). Older age was suggested to be related to lower sero-protection with marginal significance in the crude model. This relationship appeared significant in Model 2, which involved age, HbA1c level and BMI (trend P = 0.033). In addition, subjects with the highest HbA1c level (≥7.6%) tended to show a lower sero-protection proportion (crude OR, 0.39; 95% CI, 0.06–2.42) than subjects with the lowest HbA1c level (<6.5%), although this difference was not significant. After adjusting for potential confounders, we found that a higher HbA1c was independently associated with lower sero-protection with marginal significance (Model 1: trend P = 0.071; Model 2: trend P = 0.074). In addition, subjects with lower BMI showed a decreased OR for sero-protection (trend P = 0.079).

These findings suggested that (1) older age may be related to poorer antibody response as reflected in post-vaccination GMT and sero-protection rate, (2) lower BMI seemed to be associated with lower sero-response and sero-protection, and (3) higher HbA1c level might have affected immune response, showing lower ORs for sero-response and sero-protection.

To explore these findings in more detail, we conducted stratified analyses. In the analyses in which effects of age and HbA1c (**Table 5**, A), and effects of BMI and HbA1c level (**Table 5**, B) were examined, higher HbA1c still induced lower immune response, although significant relationships could be detected in only part of the trends. Particularly among older patients (≥61 y), higher HbA1c was significantly associated with lower GMT ratio (GMTR), fold rise, and sero-protection proportion (P = 0.043, P = 0.044, and P = 0.043 for each). Similar relationships were also suggested among patients with higher BMI (≥23.5 kg/m^2).

Discussion

The influenza A(H1N1)pdm09 virus was reported to be distinct from seasonal human A(H1N1).[8] The pre-vaccination antibody titer of every subject we analyzed was <1:40 in the present study. This situation facilitated the evaluation of immunogenicity. We showed that a single 15-μg dose of unadjuvanted A(H1N1) pdm09 vaccine induced sufficient antibody among patients with DM. This immunity was sufficient to meet the international criteria of the European Agency for the Evaluation of Medical Products and the US Food and Drug Administration. However, the sero-protection proportion among subjects (73%) was slightly lower than reported proportions in healthy adults (79–95%).[7,9,10] In particular, the proportion among patients >65-y-old (58%) was rather lower than the reported proportions in age-matched healthy adults (79–80%).[9,10] No serious adverse effects were observed and all reported adverse reactions were self-limited.

This study also investigated factors that may affect immunogenicity of the vaccination. We found that the following

factors might have induced lowered immunogenicity—older age, lower BMI, and higher HbA1c level. Decreased immune response in the elderly has been reported in previous studies of A(H1N1) pdm09 vaccine[10-12] and seasonal influenza vaccine.[13-15] Compromised nutritional status[16-18] and decreased T-cell activity[18-20] could be contributing factors for that finding. One study in patients with hepatitis C reported a decreasing effect of lower BMI on immune response to A(H1N1)pdm09 vaccine.[11] Another study in the elderly reported that a combination of BMI ≤ 18.5 kg/m^2 and loss of more than 5% of body weight in 6 mo found to be significantly associated with poor immune response.[21] Although the mechanisms remain unclear, nutritional status and physical strength might be involved in decreased immune response.

To the best of our knowledge, no studies have reported the effects of HbA1c on immune response. Originally, few clinical trials examined the efficacy of influenza vaccination in patients with DM. One previous study suggested an impaired immune response in patients with poorly controlled diabetes.[22] Another study in patients with well-controlled diabetes showed that humoral and cell-mediated immune responses to seasonal influenza vaccine were normal and immune response did not differ from those observed in age-matched normal subjects.[23] A third study showed that diabetic patients in the older age range or with longer disease duration showed a lower sero-conversion proportion with A(H1N1)pdm09 vaccine.[24] Such results are broadly consistent with our findings that immunity was sufficient as a whole, but older age, decreased BMI and increased HbA1c level were associated with poor immunogenicity. The reason for these minor differences is unclear, but differences in the severities of DM and the comorbidities or genetic characteristics of the population may have been involved. In stratified analysis, older patients with poorer HbA1c showed rather lower immune response. Although the sample size of the present study was small, the results might be useful in addressing this point. Larger studies are needed to confirm the present findings.

Table 2. Immuno responses to monovalent 2009 influenza A(H1N1) vaccine among diabetic patients (continu

		Geometric mean*		Fold rise*	Postvac titer**	
					≥4-fold rise	≥1:40
Category	N	Pre vac	Post vac		n (%)	n (%)
Entire sample	48	6	53	9 ($P < 0.0001$)	38 (79)	35 (73)
Sex						
Male	36	6	44	8 ($P < 0.0001$)	28 (78)	25 (69)
Female	12	7	95	13($P < 0.0001$)	10 (83)	10 (83)
		($P = 00.101$)	($P = 00.124$)	$P = 0.254$)	($P = 00.682$)	($P = 00.348$)
Age						
<57	15	8	96	12($P < 0.0001$)	12 (80)	13 (87)
57–64	14	6	62	11($P < 0.0001$)	12 (86)	11 (79)
65+	19	5	30	6($P < 0.0001$)	14 (74)	11 (58)
		($P = 0.021$)	($P = 0.027$)	($P = 0.3201$)	($P = 0.624$)	($P = 0.059$)
DM subtype						
Type 1	7	6	54	9($P = 0.0034$)	5 (71)	5 (71)
Type 2	41	6	53	9($P < 0.0001$)	33 (80)	30 (73)
		($P = 0.812$)	($P = 0.870$)	($P = 1.000$)	($P = 0.585$)	($P = 0.924$)
Duration of disease (y)						
<10	24	6	55	9 (P < 0.0001)	19 (79)	17 (71)
10+	24	6	52	9 (P < 0.0001)	19 (79)	18 (75)
		($P = 0.132$)	($P = 0.900$)	($P = 0.505$)	($P = 1.000$)	($P = 0.745$)
HbA1c (%)						
<6.5	10	6	65	11 ($P = 0.0007$)	8 (80)	8 (80)
6.5–7.5	20	6	61	11(P < 0.0001)	17 (85)	16 (80)
7.6+	18	7	42	6 (P < 0.0001)	13 (72)	11 (61)
		($P = 0.115$)	($P = 0.243$)	($P = 0.198$)	($P = 0.529$)	($P = 0.222$)
Body mass index (kg/m2)						
<22.1	13	6	34	6 ($P = 0.0006$)	8 (62)	9 (69)
22.1–23.8	16	6	50	9 (P < 0.0001)	11 (69)	10 (63)
23.9+	19	6	77	12 (P < 0.0001)	19 (100)	16 (84)
		($P = 0.806$)	($P = 0.454$)	($P = 0.221$)	($P = 0.006$)	($P = 0.296$)
Treatment of DM						
No medication	5	6	70	12 ($P = 0.0491$)	4 (80)	4 (80)
Internal use only	16	7	50	7 (P < 0.0001)	11 (69)	10 (63)
Insulin +	27	6	53	9 (P < 0.0001)	23 (85)	21 (78)
		($P = 0.472$)	($P = 0.846$)	($P = 0.674$)	($P = 0.410$)	($P = 0.649$)
Insulin-	21	7	54	8 (P < 0.0001)	15 (71)	14 (67)
Insulin+	27	6	53	9 (P < 0.0001)	23 (85)	21 (78)
		($P = 0.319$)	($P = 0.841$)	($P = 0.624$)	($P = 0.244$)	($P = 0.390$)

*Wilcoxon signed–rank test for intra-category comparisons, and the Wilcoxon rank sum test for inter-category comparisons. **χ^2 test between 2 categories and the Mantel-extension method for trend test among 3 categories.

Table 2. Immuno responses to monovalent 2009 influenza A(H1N1) vaccine among diabetic patients (continued)

		Geometric mean*		Fold rise*	Postvac titer**	
					≥4-fold rise	≥1:40
Category	N	Pre vac	Post vac		n (%)	n (%)
Treatment of hypertension						
None	27	6	54	9 (P < 0.0001)	21 (78)	20 (74)
Received	21	6	52	9 (P < 0.0001)	17 (81)	15 (71)
		(*P* = 0.580)	(*P* = 0.882)	(*P* = 0.875)	(*P* = 0.788)	(*P* = 0.838)
Treatment of hyperlipidemia						
None	31	6	57	9 (P < 0.0001)	24 (77)	24 (77)
Received	17	6	47	8 (P < 0.0001)	14 (82)	11 (65)
		(*P* = 0.457)	(*P* = 0.428)	(*P* = 0.914)	(*P* = 0.687)	(*P* = 0.343)
Prevaccination titer						
<1:10	37	5	45	9 (P < 0.0001)	30 (81)	26 (70)
1:10–1:20	11	12	97	8 (*P* = 0.0004)	8 (73)	9 (82)
		(*P* <0.0001)	(*P* = 0.169)	(*P* = 0.321)	(*P* = 0.549)	(*P* = 0.449)

*Wilcoxon signed–rank test for intra-category comparisons, and the Wilcoxon rank sum test for inter-category comparisons. **χ^2 test between 2 categories and the Mantel-extension method for trend test among 3 categories.

Better methods to improve immunogenicity among subjects with poor immune response clearly need to be considered. A second vaccination might be effective. Previous studies[25-29] have suggested that 2 doses of vaccine are required to elicit a protective immune response in populations that are immunologically naïve to a new influenza strain. One study[10] reported that immune responses in the elderly could be substantially boosted by a second dose of vaccine—among subjects ≥61-y-old who received a 15-μg dose of unadjuvanted A(H1N1)pdm09 vaccine, the sero-protection proportion was 79.1% at 21 d after the first dose, and 93.3% at 14 d after the second dose (35 d after first dose). According to our data, the proportion at 21 d after vaccination among diabetic patients >65-y-old was substantially lower (58%) and a second dose might improve immunogenicity. In addition, adjuvants might be of help. Adjuvants are used to augment cellular and humoral responses by attracting greater numbers of antigen-presenting cells to the vaccination site. According to another study[30] that performed a randomized trial in healthy adults and older individuals to evaluate the immunogenicity and safety of A(H1N1)pdm09 vaccine at varying dosages of hemagglutinin with and without adjuvants, GMTs were higher in the adjuvanted groups than with the 15-μg unadjuvanted group in both age groups. And only 61% of participants achieved sero-protection after a single dose of the 15-μg unadjuvanted vaccination, whereas 81% achieved this state after a single dose of the 7.5-μg adjuvanted vaccination. The proportion increased to 94% after a second dose in the adjuvanted group, but remained low (68%) in the unadjuvanted group. Another study that performed a similar randomized trial of A(H1N1)pdm09 vaccine also reported that the addition of MF59 adjuvant to the vaccine increased the speed and magnitude of antibody response.[31] A second vaccination with adjuvants might thus represent a better method for diabetic subjects with poor immune response. Further studies are needed to verify this possibility.

Several limitations must be considered when interpreting the results of this study. First, the investigation was conducted in a single university hospital. Our study population comprised relatively well-controlled, adequately nourished patients without serious comorbidities, which may well have influenced the generalizability of the results. In addition, the number of subjects was small, limiting the study power. Second, we evaluated immunogenicity by antibody response only. Cell-mediated immunity to A(H1N1)pdm09 vaccine also needs to be investigated to elucidate the mechanisms of diminished response among patients with older age, lower BMI or higher HbA1c level. Finally, serum samples were collected only twice (before and 3 wk after vaccination) and we did not measure serum antibody responses after the pandemic season. We therefore had no data regarding the subsequent maintenance of immunogenicity, which represents a shortcoming of this study. In addition, we were unable to monitor subjects for clinical occurrences of influenza infection or influenza-like illness during the year. We were therefore unable to evaluate the actual effects of A(H1N1)pdm09 vaccine in protecting subjects. This represents another limitation of the current study. However, this is the first report on the effects of HbA1c on immune response to influenza A(H1N1)pdm09 vaccine. The independent negative effect of HbA1c we showed in this study is noteworthy to promote awareness regarding the potential for low immunogenicity in patients with poorly controlled diabetes.

In conclusion, we found that a single dose of A(H1N1)pdm09 vaccine safely induced a sufficient level of immunity and met international criteria in patients with DM. No severe adverse events were encountered. However, older age, lower BMI, and higher HbA1c were associated with reduced immune responses. Our results showed that older patients with higher HbA1c levels should be followed particularly carefully. Further studies are needed to clarify the mechanisms involved. To minimize influenza-related morbidity and mortality in the case of future influenza pandemics, it is important to determine the most effective methods for developing protective titers among patients with DM.

Patients and Methods

Study subjects

Study subjects were 49 patients with DM who visited the department of diabetes at Osaka City University Hospital for clinical follow-up. All subjects provided written informed

Table 3. Association between selected characteristics and sero-response proportion (≥4-fold rise)

			Crude analysis			Multivariate model*		
Category	N	n (%)	OR	(95%CI)	*P* value	OR	(95% CI)	*P* value
Sex								
Male	36	28 (78)	1.00			1.00		
Female	12	10 (83)	1.43	(0.26–7.89)	0.683	9.28	(0.22–390)	0.243
Age								
<57	15	12 (80)	1.00			1.00		
57–64	14	12 (86)	0.50	(0.21–10.6)	0.685	0.28	(0.01–6.12)	0.417
65+	19	14 (74)	0.70	(0.14–3.56)	0.667	0.63	(0.04–9.79)	0.738
			(Trend *P* = 0.622)			(Trend *P* =0.666)		
Type								
Type 1	7	5 (71)	1.00			1.00		
Type 2	41	33 (80)	1.65	(0.27–10.1)	0.588	2.59	(0.05–149)	0.645
Duration of disease (years)								
<10	24	19 (79)	1.00			1.00		
10+	24	19 (79)	1.00	(0.25–4.03)	1.000	0.48	(0.04–5.92)	0.567
HbA1c (%)								
<6.5	10	8 (80)	1.00			1.00		
6.5–7.5	20	17 (85)	1.42	(0.20–10.2)	0.730	0.97	(0.06–15.3)	0.985
7.6+	18	13 (72)	0.65	(0.10–4.18)	0.650	0.13	(0.005–3.51)	0.223
			(Trend *P* = 0.527)			(Trend *P* = 0.243)		
Body mass index (kg/m2)								
<22.1	13	8 (62)	1.00			1.00		
22.1–23.8	16	11 (69)	1.38	(0.30–6.40)	0.685	4.30	80.35–52.6)	0.254
23.9+	19	19 (100)	N.A.			N.A.		
			(Trend *P* =0.012)			(Trend *P* =0.008)		
Treatment of DM								
Insulin-	21	15 (71)	1.00			1.00		
Insulin+	27	23 (85)	1.52	(0.57–4.06)	0.409	4.22	(0.26–70.3)	0.316
Prevaccination titer								
<1:10	37	30 (81)	1.00			1.00		
1: 10–1:20	11	8 (73)	0.62	(0.13–2.96)	0.551	0.26	(0.01–6.02)	0.403

Logistic regression model. *Model included all variables in the table.

consent after the nature and possible consequences of the study had been explained. The study protocol was approved by the ethics committee at Osaka City University Graduate School of Medicine and was performed in accordance with the Declaration of Helsinki. None of the applicants met the exclusion criteria for eligibility, including history of 2009 influenza A (H1N1) infection, acute febrile illness or signs of severe acute illness at the time of vaccination, history of anaphylaxis due to vaccine components, or other condition contraindicating vaccination. In November 2009, subjects were administered a single dose of monovalent inactivated unadjuvanted split-virus 2009 pH1N1 vaccine containing 15 μg/0.5 mL of hemagglutinin antigen (Lot. HP01A; BIKEN). The vaccine contained thimerosal. The seed virus was prepared from reassortant vaccine virus A/California/7/2009 NYMC X-179A (New York Medical College), distributed by the Centers for Disease Control and Prevention in the United States. The vaccine was prepared in embryonated chicken eggs using standard methods for the production of seasonal trivalent inactivated vaccine.

Information collection

Before vaccination, subjects completed a self-administered questionnaire asking about sex, age at vaccination, date of birth, and comorbid diseases. In addition, one of the investigators extracted the following patient background and clinical information from the medical records—height, weight, DM subtype, treatment for DM; hypertension, hyperlipidemia,

Table 4. Association between selected characteristics and sero-protection proportion (titer ≥ 1:40)

			Crude analysis			Multivariate model1*			Multivariate model2**		
Category	N	n(%)	OR	(95%CI)	*P* value	OR	(95%CI)	*P* value	OR	(95%CI)	*P* value
Sex											
Male	36	25 (69)	1.00			1.00					
Female	12	10 (83)	2.20	(0.41–11.8)	0.356	1.74	(0.12–24.4)	0.681			
Age											
<57	15	13 (87)	1.00			1.00			1.00		
57-64	14	11 (79)	0.56	(0.08–4.01)	0.567	0.28	(0.02–3.86)	0.343	0.22	(0.02–2.24)	0.202
65+	19	11 (58)	0.21	(0.04–1.21)	0.081	0.16	(0.02–1.57)	0.116	0.09	(0.01–0.77)	0.028
			(Trend *P* = 0.066)			(Trend *P* = 0.137)			(Trend *P*=0.033)		
Type 1	7	5 (71)	1.00			1.00					
Type 2	41	30 (73)	1.09	(0.18–6.47)	0.924	1.10	(0.04–28.0)	0.956			
Duration of disease (years)											
<10	24	17 (71)	1.00			1.00					
10+	24	18 (75)	1.24	(0.35–4.43)	0.746	1.13	(0.21–6.12)	0.886			
HbA1c (%)											
<6.5	10	8 (80)	1.00			1.00			1.00		
6.5–7.5	20	16 (80)	1.00	(0.15–6.67)	1.000	0.93	(0.09-–9.32)	0.948	1.22	(0.15–10.1)	0.853
7.6+	18	11 (61)	0.39	(0.06–2.42)	0.313	0.10	(0.007–1.45)	0.091	0.16	(0.02–1.57)	0.115
			(Trend *P* =0.224)			(Trend *P* =0.071)			(Trend *P* =0.074)		
Body mass index (kg/m²)											
<22.1	13	9 (69)	1.00			1.00			1.00		
22.1-23.8	16	10 (63)	0.74	(0.16–3.50)	0.705	1.27	(0.17–9.24)	0.815	1.02	(0.17–6.16)	0.980
23.9+	19	16 (84)	2.37	(0.43–13.0)	0.321	9.17	(0.85–99.2)	0.068	7.15	(0.84–60.6)	0.071
			(Trend *P* =0.295)			(Trend *P* =0.078)			(Trend *P* =0.079)		
Treatment of DM											
Insulin-	21	14 (67)	1.00			1.00					
Insulin+	27	21 (78)	1.75	(0.49–6.31)	0.393	1.69	(0.25–11.6)	0.592			
Prevaccination titer											
<1:10	37	26 (70)	1.00			1.00					
1: 10-1:20	11	9 (82)	1.90	(0.35–10.3)	0.454	2.92	(0.25–34.8)	0.397			

Logistic regression model. *Model included all variables in the table. **Model included age, HbA1c and BMI.

steroid use, and laboratory data. We determined HbA1c levels using a Japan Diabetes Society (JDS)-certified method.

Serum collection and antibody titer measurement

Serum samples were collected twice: before vaccination and 3 wk after vaccination. Serum antibody titer against the vaccine strain was measured using the HAI assay according to standard methods using chicken erythrocytes.[10,32] All samples were assayed at the same time at the Surveillance Center Research Institute for Microbial Disease at Osaka University at April 2010.

Statistical analysis

We analyzed 48 patients, excluding 1 patient with suspected infection

To compare baseline characteristics between patients with type 1 and type 2 DM, we used the chi-square test for categorical variables and Wilcoxon rank-sum test for continuous variables. For assessment of the immunogenicity of influenza vaccine, the following outcomes were calculated—GMT, GMTR, fold rise, sero-response proportion (≥4-fold rise), and sero-protection proportion (post-vaccination titer ≥ 1:40). A titer <1:10 was regarded as 1:5 for the purpose of calculations. Reciprocal antibody titers were analyzed after logarithmic transformation. All results are presented in the original scale by calculating the antilogarithm.

Data were stratified for analysis by sex, age (<57 y, 57–64 y, or ≥65 y), DM subtype, duration of DM (<10 y or ≥10 y), HbA1c level (<6.5%, 6.5–7.5%, or ≥7.6%), body mass index (BMI) (<22.1 kg/m^2, 22.1–23.8 kg/m^2, or ≥23.9 kg/m^2), treatment of DM (no medication, internal use only or insulin

Table 5.

A. Stratified immunogenicity analysis by age and HbA1c					
				seroresponse**	seroprotection**
HbA1c	N	GMTR*	Fold rise*	n (%)	n (%)
Age < 61					
<6.5	4	12.3	7	3(75)	3(75)
6.5–7.5	9	33.9	17	8(89)	8(89)
7.6+	9	17.8	9	7(78)	8(89)
		P = 0.390	*P* = 0.421	*P* = 0.947	*P* = 0.573
Age ≥ 61					
<6.5	6	30.8	16	5(83)	5(83)
6.5-7.5	11	11.5	8	9(82)	8(73)
7.6+	9	4.2	4	6(67)	3(33)
		P = 0.043	*P* = 0.044	*P* = 0.427	*P* = 0.043
B. Stratified immunogenicity analysis by BMI and HbA1c					
				seroresponse**	seroprotection**
HbA1c	N	GMTR*	Fold rise*	n (%)	n (%)
BMI<23.5					
<6.5	6	11.0	6	4 (67)	4 (67)
6.5–7.5	11	23.8	10	9 (82)	8 (73)
7.6+	6	7.3	4	2 (33)	3 (50)
		P = 0.379	*P* = 0.364	*P* = 0.236	*P* = 0.553
BMI ≥ 23.5					
<6.5	4	42.0	32	4 (100)	4 (100)
6.5–7.5	9	18.9	12	8 (89)	8 (89)
7.6+	12	12.8	8	11 (92)	8 (67)
		P = 0.061	*P* = 0.084	*P* = 0.723	*P* = 0.109

*Kruskal–Wallis test. **Mantel–extension method.

use), treatment of hypertension (no medication or internal use), treatment of hyperlipidemia (no medication or internal use), and prevaccination titer (<1:10 or 1:10–1:20). The significances of GMT, GMTR, and fold rise within a category were assessed using the Wilcoxon signed–rank test, and intercategory comparisons were made using the Wilcoxon rank–sum test or Kruskal–Wallis test. The chi-square test and Mantel-extension trend test were also used where appropriate.

In addition, we calculated OR and 95% CI using logistic regression modeling to evaluate the independent effects of potential confounders. The models were constructed using sero-response or sero-protection as the dependent variable, and potential predictors such as sex, age, DM subtype, duration of DM, HbA1c level, BMI, treatment of DM and prevaccination titer as explanatory variables. From these models, we also constructed a reduced model using potential predictors (age, HbA1c level, and BMI) which showed *P* values or trend *P* values < 0.2 as explanatory variables. The sero-response proportion among patients with highest BMI (≥23.9) was 100%, which was why we used the group with lowest BMI (<22.1) as the reference stratum.

Two-sided *P* values less than 0.05 were considered significant. All analyses were performed using SAS version 9.3 software (SAS Institute).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Smith SA, Poland GA. Use of influenza and pneumococcal vaccines in people with diabetes. Diabetes Care 2000; 23:95-108; PMID:10857977; http://dx.doi.org/10.2337/diacare.23.1.95
2. Simonsen L, Fukuda K, Schonberger LB, Cox NJ. The impact of influenza epidemics on hospitalizations. J Infect Dis 2000; 181:831-7; PMID:10720501; http://dx.doi.org/10.1086/315320
3. Muller LM, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AI, Rutten GE. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 2005; 41:281-8; PMID:16007521; http://dx.doi.org/10.1086/431587
4. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 1997; 46(RR-9):1-25; PMID:9148134
5. Smith SA, Poland GA; American Diabetes Association. Influenza and pneumococcal immunization in diabetes. Diabetes Care 2004; 27(Suppl 1):S111-3; PMID:14693944; http://dx.doi.org/10.2337/diacare.27.2007.S111
6. New influenza A (H1N1) virus: global epidemiological situation, June 2009. Wkly Epidemiol Rec 2009; 84:249-57; PMID:19537358.
7. Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, Dawson G, Hu W, Leggio C, Washington D, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. N Engl J Med 2009; 361:2405-13; PMID:19745216; http://dx.doi.org/10.1056/NEJMoa0907413
8. Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, Sessions WM, Xu X, Skepner E, Deyde V, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science 2009; 325:197-201; PMID:19465683; http://dx.doi.org/10.1126/science.1176225
9. Liang XF, Wang HQ, Wang JZ, Fang HH, Wu J, Zhu FC, Li RC, Xia SL, Zhao YL, Li FJ, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2010; 375:56-66; PMID:20018364; http://dx.doi.org/10.1016/S0140-6736(09)62003-1
10. Zhu FC, Wang H, Fang HH, Yang JG, Lin XJ, Liang XF, Zhang XF, Pan HX, Meng FY, Hu YM, et al. A novel influenza A (H1N1) vaccine in various age groups. N Engl J Med 2009; 361:2414-23; PMID:19846844; http://dx.doi.org/10.1056/NEJMoa0908535
11. Ohfuji S, Fukushima W, Tamori A, Maeda K, Maeda A, Hirota Y. Immunogenicity of influenza A(H1N1)pdm09 vaccine and the associated factors on lowered immune response in patients with hepatitis C. Influenza Other Respir Viruses 2013; 7:456-65; PMID:22897938; http://dx.doi.org/10.1111/j.1750-2659.2012.00424.x
12. Kao TM, Hsieh SM, Kung HC, Lee YC, Huang KC, Huang LM, Chang FY, Wang NC, Liu YC, Lee WS, et al. Immune response of single dose vaccination against 2009 pandemic influenza A (H1N1) in the Taiwanese elderly. Vaccine 2010; 28:6159-63; PMID:20659517; http://dx.doi.org/10.1016/j.vaccine.2010.07.026
13. Hui SL, Chu LW, Peiris JS, Chan KH, Chu D, Tsui W. Immune response to influenza vaccination in community-dwelling Chinese elderly persons. Vaccine 2006; 24:5371-80; PMID:16713661; http://dx.doi.org/10.1016/j.vaccine.2006.04.032
14. Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine 2006; 24:1159-69; PMID:16213065; http://dx.doi.org/10.1016/j.vaccine.2005.08.105
15. Fülöp T Jr., Wagner JR, Khalil A, Weber J, Trottier L, Payette H. Relationship between the response to influenza vaccination and the nutritional status in institutionalized elderly subjects. J Gerontol A Biol Sci Med Sci 1999; 54:M59-64; PMID:10051856; http://dx.doi.org/10.1093/gerona/54.2.M59
16. Bellei NC, Carraro E, Castelo A, Granato CF. Risk factors for poor immune response to influenza vaccination in elderly people. The Brazilian journal of infectious diseases: an official publication of the Brazilian Society of Infectious Diseases 2006; 10:269-73.
17. Hara M, Tanaka K, Hirota Y. Immune response to influenza vaccine in healthy adults and the elderly: association with nutritional status. Vaccine 2005; 23:1457-63; PMID:15670881; http://dx.doi.org/10.1016/j.vaccine.2004.09.022
18. Deng Y, Jing Y, Campbell AE, Gravenstein S. Age-related impaired type 1 T cell responses to influenza: reduced activation ex vivo, decreased expansion in CTL culture in vitro, and blunted response to influenza vaccination in vivo in the elderly. J Immunol 2004; 172:3437-46; PMID:15004143
19. Kang I, Hong MS, Nolasco H, Park SH, Dan JM, Choi JY, Craft J. Age-associated change in the frequency of memory CD4+ T cells impairs long term CD4+ T cell responses to influenza vaccine. J Immunol 2004; 173:673-81; PMID:15210831
20. Saurwein-Teissl M, Lung TL, Marx F, Gschösser C, Asch E, Blasko I, Parson W, Böck G, Schönitzer D, Trannoy E, et al. Lack of antibody production following immunization in old age: association with CD8(+)CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. J Immunol 2002; 168:5893-9; PMID:12023394
21. Sagawa M, Kojimahara N, Otsuka N, Kimura M, Yamaguchi N. Immune response to influenza vaccine in the elderly: association with nutritional and physical status. Geriatr Gerontol Int 2011; 11:63-8; PMID:20738411; http://dx.doi.org/10.1111/j.1447-0594.2010.00641.x
22. Delespesse G, Duchateau J, Bastenie PA, Lauvaux JP, Collet H, Govaerts A. Cell-mediated immunity in diabetes mellitus. Clin Exp Immunol 1974; 18:461-7; PMID:4283119
23. Pozzilli P, Gale EA, Visalli N, Baroni M, Crovari P, Frighi V, Cavallo MG, Andreani D. The immune response to influenza vaccination in diabetic patients. Diabetologia 1986; 29:850-4; PMID:3569690; http://dx.doi.org/10.1007/BF00870139
24. Nam JS, Kim AR, Yoon JC, Byun Y, Kim SA, Kim KR, Cho S, Seong BL, Ahn CW, Lee JM. The humoral immune response to the inactivated influenza A (H1N1) 2009 monovalent vaccine in patients with Type 2 diabetes mellitus in Korea. Diabetic medicine: a journal of the British Diabetic Association 2011; 28:815-7.
25. Englund JA, Walter EB, Gbadebo A, Monto AS, Zhu Y, Neuzil KM. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. Pediatrics 2006; 118:e579-85; PMID:16950949; http://dx.doi.org/10.1542/peds.2006-0201
26. Neuzil KM, Jackson LA, Nelson J, Klimov A, Cox N, Bridges CB, Dunn J, DeStefano F, Shay D. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5-8-year-old children. J Infect Dis 2006; 194:1032-9; PMID:16991077; http://dx.doi.org/10.1086/507309
27. Parkman PD, Hopps HE, Rastogi SC, Meyer HM Jr. Summary of clinical trials of influenza virus vaccines in adults. J Infect Dis 1977; 136(Suppl):S722-30; PMID:606797; http://dx.doi.org/10.1093/infdis/136.Supplement_3.S722
28. Sencer DJ, Millar JD. Reflections on the 1976 swine flu vaccination program. Emerg Infect Dis 2006; 12:29-33; PMID:16494713; http://dx.doi.org/10.3201/eid1201.051007
29. Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med 2006; 354:1343-51; PMID:16571878; http://dx.doi.org/10.1056/NEJMoa055778
30. Jackson LA, Chen WH, Stapleton JT, Dekker CL, Wald A, Brady RC, Edupuganti S, Winokur P, Mulligan MJ, Keyserling HL, et al. Immunogenicity and safety of varying dosages of a monovalent 2009 H1N1 influenza vaccine given with and without AS03 adjuvant system in healthy adults and older persons. J Infect Dis 2012; 206:811-20; PMID:22782949; http://dx.doi.org/10.1093/infdis/jis427
31. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, Stephenson I. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. N Engl J Med 2009; 361:2424-35; PMID:19745215; http://dx.doi.org/10.1056/NEJMoa0907650
32. Hirst GK. The Quantitative Determination of Influenza Virus and Antibodies by Means of Red Cell Agglutination. J Exp Med 1942; 75:49-64; PMID:19871167; http://dx.doi.org/10.1084/jem.75.1.49

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Infliximab and/or immunomodulators inhibit immune responses to trivalent influenza vaccination in adults with inflammatory bowel disease

Yoshie Hagihara[a], Satoko Ohfuji[b], Kenji Watanabe[a,*], Hirokazu Yamagami[a], Wakaba Fukushima[b], Kazuhiro Maeda[c], Noriko Kamata[a], Mitsue Sogawa[a], Masatsugu Shiba[a], Tetsuya Tanigawa[a], Kazunari Tominaga[a], Toshio Watanabe[a], Yasuhiro Fujiwara[a], Yoshio Hirota[b], Tetsuo Arakawa[a]

[a] *Department of Gastroenterology, Osaka City University Graduate School of Medicine, Japan*
[b] *Department of Public Health, Osaka City University Graduate School of Medicine, Japan*
[c] *Research Foundation for Microbial Diseases of Osaka University, Japan*

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KEYWORDS
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Abstract

Background and aims: Appropriate influenza vaccination is important for patients with inflammatory bowel disease under immunosuppressive therapy. The purpose of this study was to evaluate the influence of immunosuppressive therapy on the immune response to the trivalent influenza vaccine in adult patients with inflammatory bowel disease.
Methods: In this cohort study, 91 participants received a single dose of influenza vaccine for the 2010/2011 season. Serum samples were collected at 3 different times (pre-vaccination, 3 weeks post-vaccination, and after flu season) to measure hemagglutination inhibition antibody titers. Immune responses were compared based on immunosuppressive therapy.
Results: Among the 88 subjects who completed the study, the influenza vaccine induced a more than 4-fold increase in the mean antibody level for all flu strains. The overall seroprotection proportion (post-vaccination titer $\geq$ 1:40) was 81% for H1N1, 61% for H3N2, and 86% for B. Treatment with an immunomodulator reduced the immune response to the H1N1 strain (OR = 0.20, p = 0.01), and treatment with infliximab reduced the immune response to the

Abbreviations: AZA, azathioprine; CAI, clinical activity index; CD, Crohn's disease; CDAI, Crohn's disease activity index; CI, confidence interval; GMT, geometric mean titer; HAI, hemagglutination inhibition; IFX, infliximab; IS, group immunosuppressive group; 6MP, 6-mercaptopurine; NIS, group non-immunosuppressive group; OR, odds ratio; SD, standard deviation; UC, ulcerative colitis; WHO, World Health Organization.

* Corresponding author at: Department of Gastroenterology, Graduate School of Medicine, Osaka City University, 1-4-3, Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan. Tel.: +81 6 6645 3811; fax: +81 6 6645 3813.
E-mail address: kenjiw@med.osaka-cu.ac.jp (K. Watanabe).

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other strains (H3N2 strain: OR = 0.37, $p = 0.02$; B strain: OR = 0.18, $p = 0.03$). Combination therapy with azathioprine/6-mercaptopurine and infliximab significantly inhibited the immune response to H1N1 (OR = 0.056, $p = 0.02$).
Conclusions: Infliximab and/or immunomodulators inhibit immune responses to some strains of trivalent influenza vaccination in adults with inflammatory bowel disease. For optimization of the trivalent influenza vaccination for patients with adult inflammatory bowel disease treated with immunosuppressive agents, establishing an effective vaccination method is crucial.

1. Introduction

Inflammatory bowel disease (IBD), ulcerative colitis (UC), and Crohn's disease (CD) are accompanied by chronic inflammation of the gastrointestinal tract due to a complex interplay between environmental factors, dysregulated immune systems, and genetic susceptibility.[1] Immunosuppressive (IS) therapeutics such as immunomodulators or anti-tumor necrosis factor-α (TNF-α) agents are frequently used as aggressive therapies for IBD. However, immunosuppressive agents such as systemic corticosteroids, azathioprines (AZA)/6-mecaptopurine (6-MP), tacrolimus, methotrexate, and anti-TNF-α agents (e.g., infliximab [IFX]) increase the risk for more frequent and severe infections in IBD patients.[2–4] Combination therapies using more than one IS agent are especially associated with increased risk for opportunistic infections,[5] including bacterial and many severe and fatal viral infections.[6–8] Recent publications recommend more appropriate vaccination strategies for IBD patients as infection prophylaxis prior to IS therapy.[9,10]

Influenza, caused by type A or type B viruses, is a prevalent respiratory illness that can lead to other associated complications and hospitalization. Influenza patients often seek medical attention in hospital emergency rooms, and absence rates for workers and students increase dramatically during the influenza season.[11] In the US, approximately 226,000 patients are hospitalized annually for influenza, and approximately 36,000 cases of influenza-related deaths are reported each year.[12,13] In 2009, the World Health Organization (WHO) reported of the human infection with influenza A(H1N1). HIN1 spread rapidly throughout the world during the 2009/2010 influenza season, leading WHO to declare a phase 6 pandemic alert.[14] Epidemiologic studies for the pandemic outbreak in 2009 revealed that the risk of influenza-associated complications for adults infected with influenza A(H1N1)pdm09 was higher than usual for seasonal influenza.[15]

Several recent studies that examined the immunogenicity of the influenza A(H1N1)pdm09 vaccine in IBD patients[16–23] have cautioned that combination therapy with anti-TNF-α agents and immunomodulators (AZA/6MP) may reduce the immune response to vaccines.[17,18] Similar findings have been reported for the trivalent influenza vaccine, which is routinely distributed as a seasonal influenza vaccine.[16,22,24] These reports also showed that children undergoing IS therapy for IBD exhibited reduced immune response to the vaccine. To the best of our knowledge, however, no studies have reported the effect of IS therapy on the specific immune response to the individual strains covered by the trivalent influenza vaccine in adults with IBD. Although adults are generally considered to generate a better immune response to the vaccine than children do, it is important to examine the effect of IS therapy in adult IBD patients. Therefore, the aim of the present study was to investigate the immune response to the trivalent influenza vaccine in adult IBD patients undergoing IS treatments.

2. Materials and methods

2.1. Subjects

We conducted this prospective, open label, cohort study from September 2010 to July 2011 in the Department of Gastroenterology at Osaka City University Hospital. Between 29 September 2010 and 14 October 2010, IBD outpatients (minimum age, 20 years) were recruited for participation in the study.

The exclusion criteria were as follows: patient had already received 2010 trivalent inactivated influenza vaccine; patient had history of influenza infection within the last 6 months; patient had history of anaphylactic reaction to previous influenza vaccine or vaccine components or of acute febrile illness or signs of severe acute illness at the time of vaccination. All participants provided written, informed consent following a detailed explanation of the nature and possible consequences of the study. All participants in the study signed informed consent forms. We estimated the appropriate sample size was 100 participants for the present study based on the reference of the guidance of the European Committee for Proprietary Medical Products.[25] The study protocol was approved by the Ethics Review Board of the Osaka City University Graduate School of Medicine.

2.2. Data acquisition

At the time of recruitment, we obtained the following patient information from the medical records: defined disease (ulcerative colitis [UC] or Crohn's disease [CD]); disease duration; current IS therapy (corticosteroids, tacrolimus, AZA, 6-MP and IFX), which has been continued for more than 3 months; and data from blood tests (white blood cell count, differential leukocyte count, serum albumin, hematocrit, C-reactive protein). All medications were required to be stable prior to vaccination and for at least 3 weeks after vaccination. Validated clinical activity scores, clinical activity index (CAI) of Rachmilewitz index,[26] and Crohn's disease activity index (CDAI),[27,28] were used to assess disease activity in patients with UC and CD, respectively. A CAI score of ≥ 5 for UC and a CDAI score of >150 for CD were defined as active

stage, and a CAI of ≤ 4 for UC and a CDAI of ≤ 150 for CD were defined as remission stage. Participants receiving IS therapy at the time of vaccination were classified as the immunosuppressive (IS) group, and the remaining participants were considered the non-immunosuppressive (NIS) group, which included participants treated with other medications (e.g., 5-aminosalicylates).

Before vaccination, participants were asked to complete a self-administered questionnaire to collect the following information: age at vaccination, body height and weight, underlying illnesses, past medical history, and allergic history (including allergy to eggs).

2.3. Vaccination with trivalent vaccine

All participants received a single subcutaneous dose of the 2010 trivalent inactivated influenza vaccine (Lot. HA101E, BIKEN, Osaka, Japan). This vaccine included the following antigen strains: A/California/7/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008. A standard 0.5-mL dose of the vaccine contained 15 μg of the hemagglutinin antigen of each strain.

2.4. Determination of hemagglutination inhibition antibody titers

Serum samples were collected at 3 time points: before vaccination (S0), 3 weeks post-vaccination (S1) according to our previous investigation[29], and after the influenza season (S2; approximately 7 months after vaccination). All serum specimens were stored at −80 °C until used for testing for hemagglutination inhibition (HAI) antibody titers against all 3 strains. HAI antibody titers were determined using the standard microtiter HAI method with the same antigens as in the vaccine.[29,30] All samples were assayed at the laboratory of the Research Foundation for Microbial Diseases of Osaka University between July and September 2011.

2.5. Assessment of side effects

Participants were surveyed regarding the presence of related symptoms for the following side effects: ocular and respiratory symptoms within 24 h after vaccination (red eyes, facial edema, and any respiratory symptoms—coughing, wheezing, chest tightness, difficulty breathing, difficulty swallowing, and sore throat), systemic symptoms within 48 h (fever, general malaise, myalgia, headache, and rash), and local symptoms within 48 h (redness, swelling, induration, itching, and pain).

2.6. Statistical analyses

The following outcomes were calculated to assess the immunogenicity of the influenza vaccine: geometric mean titer (GMT), mean fold-rise, seroresponse proportion ($\geq$4-fold rise), and seroprotection proportion (post-vaccination titer $\geq$1:40). For data processing, titers less than 1:10 were regarded as 1:5, and reciprocal antibody titers were analyzed after logarithmic transformation. The results are presented in the original scale by calculating the antilogarithm. We also performed a stratified analysis to investigate the effect of potential confounders: age at vaccination (tertile), sex, defined disease (UC or CD), disease activity (remission or active), immunosuppressive treatment, and pre-vaccination titer (<1:10, 1:10–1:20, and $\geq$1:40). The significance of fold-rise within a category was assessed by the Wilcoxon signed-rank test, and intercategory comparisons were made using either the Wilcoxon rank-sum test or the Kruskal–Wallis test. The χ^2 test or Mantel-extension method for the trend test was also used where appropriate.

Furthermore, to consider the independent effect of individual immunosuppressive therapy for immune response, multivariate analyses were conducted using logistic regression

Table 1 Baseline characteristics of participants with inflammatory bowel disease.

	All (n = 88)
Characteristics	*n* (%)
Age at vaccination (years)	
Mean (±SD)	44.4 (±14.4)
Gender	
Male	51 (58)
Female	37 (42)
Disease	
UC	45 (51)
CD	43 (49)
Disease activity	
Remission stage	74 (84)
Active stage	14 (16)
Immunosuppressive therapy	
Not receiving(NIS group)	30 (34)
Receiving(IS group)	58 (66)
Corticosteroids	6 (7)
Tacrolimus	2 (2)
AZA/6MP	31 (35)
AZA/6MP monotherapy	21 (24)
AZA/6MP + IFX	10 (11)
IFX	33 (38)
IFX monotherapy	23 (26)
IFX + AZA/6MP	10 (11)
Pre-vaccination titer	
H1N1 < 1:10	51 (58)
1:10–1:20	20 (23)
$\geq$1:40	17 (19)
H3N2 < 1:10	53 (60)
1:10–1:20	25 (28)
$\geq$1:40	10 (11)
B < 1:10	26 (30)
1:10–1:20	29 (33)
$\geq$1:40	33 (38)

SD, standard deviation; UC, ulcerative colitis; CD, Crohn's disease. NIS group, non-immunosuppressive group; IS group, immunosuppressive group.
AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.
Data are expressed as *n* (%) of patients, unless otherwise indicated.

Table 2 Changes in the geometric mean titer for each strain of trivalent influenza vaccine during the study period.

Category	Influenza A(H1N1)			Fold rise [a]	Influenza A(H3N2)			Fold rise [a]	Influenza B			Fold rise [a]
	Geometric mean titer [a]				Geometric mean titer [a]				Geometric mean titer [a]			
	Before vaccination	After vaccination	After season	S1/S0	Before vaccination	After vaccination	After season	S1/S0	Before vaccination	After vaccination	After season	S1/S0
	(S0)	(S1)	(S2) [b]		(S0)	(S1)	(S2) [b]		(S0)	(S1)	(S2) [b]	
Total patients	11	83	30	7.7***	8.5	55	26	6.4***	21	95	44	4.6***
Tertile age at vaccination (years)												
<38	16	94	39	5.9***	8	61	30	7.4***	20	118	57	5.8***
38–48	10	108	37	11***	6.6	40	17	6.1***	26	113	53	4.3***
≥49	8	54	18	7.1***	12	69	34	5.8***	16	62	28	3.9***
	$p = .08$	$p = .11$	$p = .03$	$p = .43$	$p = .12$	$p = .30$	$p = .09$	$p = .71$	$p = .29$	$p = .16$	$p = .07$	$p = .44$
Gender												
Male	11	87	31	7.8***	8	45	21	5.9***	21	90	41	4.3***
Female	10	77	28	7.6***	10	71	36	7.3***	20	102	50	5.1***
	$p = .82$	$p = .27$	$p = .14$	$p = .99$	$p = .43$	$p = .14$	$p = .43$	$p = .71$	$p = .88$	$p = .80$	$p = .68$	$p = .73$
Disease												
UC	9	69	24	7.5***	9	75	35	8.5***	21	93	45	4.5***
CD	21	97	37	7.9***	8	39	19	4.8***	21	97	44	4.7***
	$p = .49$	$p = .17$	$p = .11$	$p = .74$	$p = .51$	$p = .06$	$p = .02$	$p = .15$	$p = .98$	$p = .60$	$p = .86$	$p = .84$
Disease activity												
Remission stage	10	76	27	7.6***	8	50	24	6.2***	19	90	$p = .38$	4.6***
Active stage	16	131	47	8.0**	11	88	38	8.0**	30	131	49	4.4**
	$p = .30$	$p = .13$	$p = .17$	$p = .89$	$p = .49$	$p = .04$	$p = .23$	$p = .53$	$p = .30$	$p = .38$	$p = .90$	$p = .74$
Immunosuppressive therapy												
−(NIS group)	10	88	33	8.4***	13	73	35	5.7***	27	90	45	3.3***
+(IS group)	11	80	28	7.4***	7	47	22	6.8***	18	98	44	5.5***
	$p = .76$	$p = .58$	$p = .61$	$p = .99$	$p = .001$	$p = .12$	$p = .045$	$p = .28$	$p = .18$	$p = .56$	$p = .99$	$p = .10$

Corticosteroids												
−	10	78	29	7.6***	8.7	47	22	5.3***	20	86	39	4.2***
+	20	180	45	9**	6.3	508	226	81**	28	403	202	14*
	$p = .20$	$p = .12$	$p = .39$	$p = .94$	$p = .28$	$p = .0004$	$p = .001$	$p = .0002$	$p = .63$	$p = .002$	$p = .002$	$p = .07$
Tacrolimus												
−	11	83	30	7.7***	8.6	56	27	6.4***	21	96	45	4.5***
+	10	80	20	8	5	28	10	5.7	7	80	20	11
	$p = .47$	$p = .97$	$p = .62$	$p = .79$	$p = .26$	$p = .52$	$p = .30$	$p = .85$	$p = .24$	$p = .69$	$p = .34$	$p = .29$
AZA/6MP												
−	12	97	32	7.8***	9.4	51	25	5.4***	21	91	43	4.3***
+	8.2	61	26	7.5***	7.2	63	28	8.7***	20	102	47	5.2***
	$p = .12$	$p = .04$	$p = .53$	$p = .94$	$p = .12$	$p = .56$	$p = .64$	$p = .099$	$p = .41$	$p = .41$	$p = .70$	$p = .76$
IFX												
−	10	82	31	8.3***	9	77	37	8.2***	25	112	55	4.4***
+	12	83	28	6.8***	7	31	15	4.2***	15	72	30	4.9***
	$p = .61$	$p = .85$	$p = .77$	$p = .67$	$p = .27$	$p = .004$	$p = .002$	$p = .09$	$p = .04$	$p = .19$	$p = .09$	$p = .73$
Pre-vaccination titer												
<1:10	5	73	21	15***	5	42	19	8.3***	5	58	23	12***
1:10–1:20	15	83	29	5.7***	12	70	30	5.7***	13	76	31	5.6***
≥1:40	74	120	77	1.6**	61	130	86	2.1**	91	170	102	1.9***
	$p < .0001$	$p = .02$	$p = .001$	$p < .0001$	$p < .0001$	$p = .002$	$p = .001$	$p < .0001$	$p < .0001$	$p = .004$	$p < .0001$	$p < .0001$

UC, ulcerative colitis; CD, Crohn's disease; NIS group, non-immunosuppressive group; IS group, immunosuppressive group; AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.

[a] Wilcoxon signed-rank test for intracategory comparisons, and either the Wilcoxon rank-sum test or the Kruskal–Wallis test for intercategory.

[b] Excluded influenza infected patients for 3 physician diagnosed influenza patients and patients(3 of H1N1 and 1 of B) with serologically diagnosed influenza.

* $p < .1$.

** $p < .05$.

*** $p < .0001$.

Table 3 Seroprotection proportion to trivalent influenza vaccine during the study period.

Category	Number with Seroprotection proportion (≥1:40), *n* (%)								
	Influenza A(H1N1)			Influenza A(H3N2)			Influenza B		
	S0	S1	S2	S0	S1	S2	S0	S1	S2
Total patients	17 (19%)	71 (81%)	41 (51%)	10 (11%)	54 (61%)	31 (37%)	33 (38%)	76 (86%)	55 (66%)
Tertile age at vaccination (years)									
<38	10 (33%)	26 (87%)	18 (69%)	3 (10%)	20 (67%)	12 (42%)	13 (43%)	29 (97%)	22 (81%)
38–48	4 (13%)	27 (90%)	16 (57%)	0 (0%)	15 (50%)	6 (21%)	13 (43%)	27 (90%)	20 (69%)
≥49	3 (11%)	18 (64%)	7 (26%)	7 (25%)	19 (68%)	13 (48%)	7 (25%)	20 (71%)	13 (48%)
		p = .03	*p* = .002	*p* = .06	*p* = .95	*p* = .70	*p* = .53	*p* = .006	*p* = .01
Gender									
Male	11 (21%)	40 (78%)	27 (55%)	5 (10%)	29 (57%)	14 (29%)	19 (37%)	42 (82%)	30 (63%)
Female	6 (16%)	31 (84%)	14 (44%)	5 (14%)	25 (68%)	17 (49%)	14 (38%)	34 (92%)	25 (71%)
	p = .65	*p* = .53	*p* = .325 (14%)	*p* = .56	*p* = .31	*p* = .06	*p* = .59	*p* = .20	*p* = .40
Disease									
UC	5 (11%)	34 (76%)	16 (37%)	5 (11%)	32 (71%)	19 (43%)	16 (36%)	40 (89%)	31 (70%)
CD	12 (28%)	37 (86%)	25 (66%)	5 (12%)	22 (51%)	12 (30%)	17 (40%)	36 (84%)	24 (62%)
	p = .048	*p* = .21	*p* = .01	*p* = .57	*p* = .06	*p* = .21	*p* = .61	*p* = .48	*p* = .39
Disease activity									
Remission stage	13 (18%)	58 (78%)	33 (49%)	8 (11%)	23 (33%)	47 (68%)	26 (35%)	63 (85%)	47 (68%)
Active stage	4 (29%)	13 (93%)	8 (62%)	2 (14%)	8 (57%)	8 (57%)	7 (50%)	13 (93%)	8 (57%)
	p = .63	*p* = .21	*p* = .39	*p* = .93	*p* = .04	*p* = .43	*p* = .50	*p* = .44	*p* = .43
Immunosuppressive therapy									
−(NIS group)	5 (17%)	25 (83%)	14 (50%)	7 (23%)	23 (77%)	13 (45%)	11 (37%)	26 (87%)	19 (66%)
+(IS group)	12 (21%)	46 (79%)	27 (51%)	3 (5%)	31 (53%)	18 (33%)	22 (38%)	50 (86%)	36 (67%)
	p = .50	*p* = .65	*p* = .94	*p* = .002	*p* = .06	*p* = .27	*p* = .02	*p* = .95	*p* = .92
Corticosteroids									
−	15 (18%)	65 (79%)	37 (49%)	10 (12%)	48 (59%)	26 (33%)	30 (37%)	70 (85%)	49 (64%)
+	2 (33%)	6 (100%)	4 (67%)	0 (0%)	6 (100%)	5 (83%)	3 (50%)	6 (100%)	6 (100%)
	p = .44	*p* = .21	*p* = .4110 (12%)	*p* = .45	*p* = .04	*p* = .01	*p* = .66	*p* = .31	*p* = .07
Tacrolimus									
−	17 (20%)	69 (80%)	41 (52%)	10 (12%)	53 (62%)	31 (38%)	33 (38%)	74 (86%)	54 (67%)
+	0 (0%)	2 (100%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	2 (100%)	1 (50%)
	p = .03	*p* = .48	*p* = .14	*p* = .51	*p* = .74	*p* = .27	*p* = .54	*p* = .57	*p* = .62
AZA/6MP									
−	13 (23%)	50 (88%)	28 (53%)	8 (14%)	35 (61%)	18 (33%)	20 (35%)	49 (86%)	35 (65%)
+	4 (13%)	21 (68%)	13 (46%)	2 (6%)	19 (61%)	13 (45%)	13 (42%)	27 (87%)	20 (69%)
	p = .36	*p* = .02	*p* = .58	*p* = .29	*p* = .99	*p* = .27	*p* = .12	*p* = .88	*p* = .70
IFX									
−	9 (16%)	44 (80%)	24 (48%)	8 (15%)	39 (71%)	24 (46%)	23 (42%)	50 (91%)	39 (75%)
+	8 (24%)	27 (82%)	17 (55%)	2 (6%)	15 (45%)	7 (22%)	10 (30%)	26 (79%)	16 (52%)
	p = .55	*p* = .83	*p* = .55	*p* = .50	*p* = .02	*p* = .03	*p* = .16	*p* = .11	*p* = .03
Pre-vaccination titer									
<1:10	0 (0%)	35 (69%)	18 (40%)	0 (0%)	28 (53%)	12 (24%)	0 (0%)	17 (65%)	11 (42%)
1:10–1:20	0 (0%)	19 (95%)	8 (40%)	0 (0%)	16 (64%)	9 (38%)	0 (0%)	26 (90%)	15 (57%)
≥1:40	17 (100%)	17 (100%)	15 (94%)	10 (100%)	10 (100%)	10 (100%)	33 (100%)c	33 (100%)	30 (94%)
	p < .0001	*p* = .003	*p* = .001	*p* < .0001	*p* = .02	*p* < .0001	*p* < .0001	*p* = .0005	*p* = .0001

UC, ulcerative colitis; CD, Crohn's disease; NIS group, non-immunosuppressive group; IS group, immunosuppressive group.
AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.
Data are expressed as *n* (%) of patients, unless otherwise indicated.
χ^2 test between 2 categories and the Mantel-extension method for trend test among 3 categories.

models with potential confounders. The models were constructed with seroprotection after vaccination as the dependent variable, and the following factors were selected as potential confounders (age, disease activity, and pre-vaccination titer) because these variables were suggested to be associated with seroprotection for at least 1 of 3 vaccine strains in the univariate analyses ($p < 0.05$). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

To assess side effects, we compared the proportion of patients with each symptom across the 2 groups (IS and NIS groups) using the χ^2 test or Fisher's exact test. All tests were 2-sided. All analyses were performed using SAS, version 9.1.3 (SAS Institute).

3. Results

3.1. Study participants

Ninety-one IBD patients received a single dose of the influenza vaccine between 29 September 2010 and 14 October 2010. The participants were followed-up until July 2011 (i.e., study period). Serum samples at S0 (before vaccination), S1 (3 weeks post-vaccination), and S2 (after influenza season) were collected from 91, 88, and 88 patients, respectively. Between S1 and S2, however, 3 subjects were diagnosed with influenza by the rapid test at a medical institution. Another 4 subjects were serologically diagnosed with influenza infection (3 with A(H1N1) and 1 with B; titer increased more than 4 times in S2 compared with S1). Thus, the data from these infected subjects were excluded from S2 analysis.

Patient characteristics are shown in Table 1. Forty-five patients had UC and 43 had CD. Five patients with UC and 9 with CD were in the active stage according to the respective disease activity index before vaccination. Fifty-eight patients were treated with immunosuppressive therapy (IS group) and the other 30 were placed into the NIS group.

3.2. Immune responses to the trivalent influenza vaccine

The immune responses (GMT, fold-rise, seroresponse proportion and, seroprotection proportion) to the trivalent influenza vaccine were calculated. Table 2 summarizes the change in the GMTs and fold-rise for each vaccine strain during the study period. In all participants, GMTs after vaccination (S1) increased to 83 (H1N1), (H3N2), and 95 (B), representing a mean fold-rise of 7.7 (H1N1), 6.4 (H3N2), and 4.6 (B), respectively. The corresponding seroresponse proportion ($\geq$4 fold-rise) was 73% (95% CI, 64–82%) for H1N1, 67% (57–77%) for H3N2, and 53% (43–63%) for B. These findings suggested that the trivalent 2010/11 seasonal influenza vaccine was immunogenic in adult IBD patients.[31] After the influenza season (S2), however, GMTs decreased to less than 50% for all 3 vaccine strains.

GMTs and fold-rise after vaccination (S1) did not differ significantly with respect to age, sex, disease, disease activity, or immunosuppressive therapy (NIS or IS group). On the other hand, patients with a higher pre-vaccination titer had higher GMTs and lower mean fold-rises (all 3 strains, $p < 0.0001$). For individual immunosuppressive therapy, participants treated with corticosteroids exhibited unexpectedly increased GMTs for H3N2 and B, whereas those treated with AZA/6-MP or with IFX had significantly decreased GMTs for H1N1 or H3N2, respectively.

Table 3 summarizes the changes in the seroprotection proportion for each vaccine strain during the study period. In all participants, vaccination increased the seroprotection proportion to 81% (73–89%) for H1N1, 61% (51–71%) for H3N2, and 86% (79–93%) for B; the proportion was slightly lower for H3N2 than for the other strains. After the influenza season (S2), these proportions decreased to 51% (40–62%) for H1N1, 37% (48–74%) for H3N2, and 66% (56–76%) for B, respectively.

In the stratified analyses, older participants exhibited significantly decreased seroprotection against H1N1 ($p = 0.03$) and B ($p = 0.006$) at S1. With respect to the clinical characteristics, participants in remission stage exhibited significantly reduced immune responses to H3N2 ($p = 0.04$). Participants treated with corticosteroids exhibited increased seroprotection especially against H3N2, whereas those treated with AZA/6-MP and those treated with IFX showed significantly decreased seroprotection against H1N1 and H3N2, respectively.

3.3. Effect of independent and combination immunosuppressive therapy on immune responses to the trivalent influenza vaccine

Data from the above-mentioned univariate assessments suggested that the immune response to the vaccine might be reduced in patients undergoing specific immunosuppressive therapy such as AZA/6MP and IFX. More significant differences were observed in the seroprotection rate than in the seroresponse rate to assess the effect of AZA/6MP or IFX. Thus, to investigate the independent effect of these treatments, multivariate analyses were carried out using the seroprotection rate (Table 4). The seroprotection proportion of all patients undergoing steroid treatment increased more than 40-fold ($\geq$1:40) at S1. Therefore, we could not add the category of steroids to the model used for the logistic regression analysis.

Even after considering the effect of potential confounders, however, participants treated with AZA/6MP exhibited significantly low ORs for seroprotection against H1N1 (OR = 0.20, $p = 0.01$). Participants treated with IFX exhibited significantly decreased ORs of seroprotection against H3N2 (OR = 0.37, $p = 0.02$) and B (OR = 0.18, $p = 0.03$).

We next performed multivariate analyses for the combination of these immunosuppressive therapies (Table 5). Participants undergoing IFX monotherapy showed significantly decreased ORs for seroprotection against H3N2 (OR = 0.13, $p = 0.01$). Combination therapy with AZA/6MP and IFX was associated with significantly decreased ORs for seroprotection against H1N1 (OR = 0.056, $p = 0.02$). Combination therapy with AZA/6MP and IFX also led to decreased ORs for the B strain; however, this finding was not statistically significant owing to the limited number of subjects analyzed. Thus, the multivariate analysis data showed that each individual drug or their combination therapy were likely to independently affect the immune response to at least 1 of the 3 influenza vaccine strains.

Table 4 Factors associated with seroprotection after trivalent influenza vaccination.

Category	Influenza A(H1N1	*P*	Influenza A(H3N2)	*P*	Influenza B	*P*
	OR (95% CI)		OR (95% CI)		OR (95% CI)	
Tertile age at vaccination (years)						
<38	1.00		1.00		1.00	
38–48	3.06 (0.45–21)	0.26	0.39 (0.11–1.31)	0.13	0.27 (0.02–3.25)	0.30
≥49	0.24 (0.24–1.41)	0.11	0.60 (0.16–2.21)	0.44	0.04 (0.003–0.56)	0.02
Disease activity						
Remission stage	1.00		1.00		1.00	
Active stage	4.44 (0.38–52)	0.24	4.01 (0.82–20)	0.09	1.96 (0.18–22)	0.59
AZA/6MP						
−	1.00		1.00		1.00	
+	0.20 (0.06–0.72)	0.01	1.64 (0.72–3.74)	0.24	1.42 (0.39–5.22)	0.60
IFX						
−	1.00		1.00		1.00	
+	1.19 (0.39–3.64)	0.76	0.37 (0.16–0.86)	0.02	0.18 (0.04–0.82)	0.03
Pre-vaccination titer						
<1:10	1.00		1.00		1.00	
1:10–1:20	8.25 (0.88–77)	0.06	1.62 (0.58–4.53)	0.36	8.92 (1.13–71)	0.04

AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.
Logistic regression model: CI, confidence interval; OR, odds ratio.
Pre-vaccination titer of ≥1:40 was excluded (Data of 71 for H1N1, 78 for H3N2, and 55 for B were analyzed.).
Model included age at vaccination (years), disease activity, AZA, IFX and pre-vaccination titer.

3.4. Side effects of the trivalent influenza vaccination

Severe side effects, including fatalities, did not occur in the present study. In addition, the disease activities of participants did not change significantly during the study period (data not shown). Table 6 summarizes the proportion of subjects who reported adverse reactions. Ocular and respiratory symptoms occurred in 11 subjects (13%) within 24 h, whereas systemic symptoms and local reactions occurred in 29 subjects (34%) and 58 subjects (67%), respectively, within 48 h. The most frequent systemic symptom (20 subjects) was general malaise (24%), and the most frequent local reaction (47 subjects) was redness (55%). Comparison of the IS group and NIS group revealed no significant difference in the frequency of the reported symptoms between the 2 groups.

4. Discussion

The recent development of immunomodulators or anti TNF-α agents has led to better clinical prognoses and outcomes for patients with IBD.[32] Efforts to control infectious diseases, including vaccination, however, have become an important issue for IBD patients undergoing these therapies.[33] Appropriate vaccinations against hepatitis B virus, pneumococcus, human papilloma virus, influenza virus, etc., prior to beginning immunosuppressive therapies, are recommended in several guidelines.[9,10,34,35] The pandemic of the influenza A(H1N1)pdm09 virus led to concerns regarding influenza vaccination in IBD patients.[36]

At least one previous study indicated that influenza vaccination did not influence IBD activity.[20] Thus, evaluation

Table 5 Inhibition for seroprotective efficacy of the trivalent influenza vaccination due to combination immunosuppressive therapy.

	Influenza A(H1N1)		*P*	Influenza A(H3N2)		*P*	Influenza B		*P*
	n (%)	OR (95% CI)		*n* (%)	OR (95% CI)		*n* (%)	OR (95% CI)	
Neither AZA/6MP nor IFX	23 (82)	1.00		19 (70)	1.00		17 (81)	1.00	
AZA/6MP monotherapy	12 (67)	0.19 (0.03–1.16)	0.07	12 (60)	0.66 (0.18–2.43)	0.53	10 (91)	6.83 (0.38–123)	0.19
IFX monotherapy	14 (88)	1.05 (0.13–8.30)	0.96	8 (36)	0.13 (0.03–0.58)	0.01	12 (75)	0.60 (0.05–6.89)	0.68
combination therapy of AZA/6MP + IFX	5 (56)	0.056 (0.005–0.62)	0.02	5 (56)	0.37 (0.07–2.14)	0.27	4 (57)	0.10 (0.01–2.15)	0.14

AZA, azathioprine; 6MP, 6-mercaptopurine; IFX, infliximab.
Logistic regression model: CI, confidence interval; OR, odds ratio.
Pre-vaccination titer of ≥1:40 was excluded (Data of 71 for H1N1, 78 for H3N2, and 55 for B were analyzed.)
Model included age at vaccination (years), disease activity, and pre-vaccination titer.

Table 6 Side effects of trivalent influenza vaccination in participants with inflammatory bowel disease.

	Total patients (n = 86)	IS group (n = 56)	NIS group (n = 30)	P
Ocular and respiratory symptoms within 24 h	11 (13%)	8 (14%)	3 (10%)	0.74
Red eyes	2 (2%)	2 (4%)	0	0.54
Facial edema	1 (1%)	1 (2%)	0	1
Any respiratory symptoms	7 (8%)	5 (9%)	2 (7%)	1
Coughing	7 (8%)	5 (9%)	2 (7%)	1
Wheezing	0	0	0	
Chest tightness	0	0	0	
Difficulty breathing	0	0	0	
Difficulty swallowing	0	0	0	
Hoarseness	2 (2%)	2 (4%)	0	0.54
Sore throat	5 (6%)	3 (5%)	2 (7%)	1
	29 (34%)	17 (31%)	12 (40%)	0.48
Systemic symptoms within 48 h	0	0	0	
Fever ($\geq$37.5 °C)	20 (24%)	12 (22%)	8 (27%)	0.61
General malaise	10 (12%)	5 (9%)	5 (17%)	0.44
Myalgia	8 (9%)	3 (5%)	5 (17%)	0.12
Headache	2 (2%)	1 (2%)	1 (3%)	1
Rash	58 (67%)	38 (68%)	20 (67%)	1
Local reaction within 48 h	47 (55%)	38 (68%)	20 (67%)	0.5
Redness	40 (47%)	29 (52%)	18 (60%)	0.37
Swelling	31 (36%)	20 (36%)	11 (37%)	1
Induration				
Itching	29 (34%)	19 (34%)	10 (33%)	1
Pain	25 (29%)	17 (30%)	8 (27%)	0.81
Medical office visit due to above symptoms	2 (2%)	1 (2%)	1 (3%)	1

NIS group, non-immunosuppressive group; IS group, immunosuppressive group.
Date are expressed as n (%) of patients, unless otherwise indicated.
χ^2 test or Fisher's exact test between 2 categories.

of immunogenicity by the trivalent influenza vaccine and optimization of the vaccination, especially in IBD patients treated with immunosuppressants, is an important next step. To date, no study has elevated the immune responses to the trivalent influenza vaccine in adult IBD patients. The findings of the present study revealed that HAI antibody titers reduced for all 3 vaccine strains after the influenza season. Influenza A and B virus variants result from frequent antigenic change (i.e., antigenic drift), which renders an individual susceptible to new strains despite previous exposure to influenza. For these reasons, the trivalent influenza vaccine must be modified according to the strain antigens, and patients must be immunized annually. Different efficacies of the trivalent influenza vaccine in IBD patients, in particular, those treated with immunosuppressive drugs, must be evaluated carefully and precisely, and the process of trivalent influenza vaccination should be optimized for these patients.

To prevent and control influenza infection and the associated complications, seroprotective levels of the antibody must be acquired before the influenza season. Evaluation of the characteristics associated with the immune response after vaccination in the stratified analyses revealed that GMT and the seroprotection proportion were significantly higher in participants treated with corticosteroids than in those not treated with corticosteroids. This finding was unexpected, because steroid treatment is known to inhibit the immune response to influenza vaccine.[37] Our results do not clarify why patients undergoing corticosteroid treatment exhibited higher antibody titers. One possibility, however, is that some patients under corticosteroid treatment might develop an asymptomatic infection between S0 and S1, as evidenced by the extremely high individual antibody titer (more than 640) in some of the study participants. Thus, this positive relationship must be cautiously interpreted.

Conversely, seroprotection proportion against H1N1 was significantly lower in participants treated with AZA/6MP (p = 0.01). In addition, treatment with IFX significantly reduced the immune response to H3N2 (p = 0.02) and B (p = 0.03), whereas combination therapy with AZA/6MP and IFX showed significant inhibitory effects on H1N1 (p = 0.02). The results of multivariate analyses showed that each drug alone or in combination therapy independently reduced the immune response to the influenza vaccine for at least 1 of the 3 influenza vaccine strains. Some studies have also reported that combination therapies with anti-TNF-α agents and immunomodulators reduces the immunogenicity of the influenza A(H1N1)pdm09 vaccine more than monotherapy with anti TNF-α agents does in adult IBD patients.[17,18] In addition, Mamula et al.[22] reported that combination therapy reduced the immunogenicity of the seasonal influenza vaccine more than monotherapy did in pediatric IBD patients. Furthermore, studies in pediatric IBD patients treated with whole immunosuppressive therapy or anti-TNF-α agents indicated that the immunogenicity of influenza B is inhibited more than that of influenza A(H1N1, H3N2).[16,24] However, thiopurines do not affect the immunogenicity of influenza B in adult IBD patients.[38] In patients with another immunologic disease – spondyloarthritis – anti-TNF-α agents can inhibit the

immunogenicity of the influenza A(H1N1)pdm09 vaccine.[39] Taken together, these findings suggest that immunosuppressive therapy independently inhibits the immunogenicity of the trivalent influenza vaccine in both pediatric and adult IBD patients.[40] Immunologic studies must be performed to evaluate the mechanism underlying the effect of these immunosuppressive therapies on the immunogenicity of vaccines in IBD patients. Investigations of host immunologic potential (i.e., T cells) and antibody formation response (i.e., B cells) are also needed.[17,41]

As for side effects, there was no significant difference in the proportion of each symptom between the IS and NIS groups. In addition, vaccination did not influence IBD activity in the participants, consistent with results of a previous study.[20] Thus, the trivalent influenza vaccination appears to be safe for adult IBD patients, regardless of whether they are receiving immunosuppressive treatment.

This is the first study to show that immunosuppressive therapy (AZA/6MP and/or IFX) inhibits the immunogenicity of the trivalent influenza vaccine in adult IBD patients. This study, however, has some limitations. We did not analyze the influence of dosage and treatment duration for each immunosuppressive drug. Further, the number of participants treated with corticosteroids or combination therapy (AZA/6MP and IFX) was relatively small, which could influence the results of statistical analysis. In addition, healthy controls were not included for comparison. However, the comparisons between the IS group and NIS group should be clinically important with reference to past notable investigations.[17,20,23] Moreover, the NIS group of IBD patients is an appropriate control group to reveal the influence of immunosuppressive therapy on immune responses to the trivalent influenza vaccine.

To optimize the trivalent influenza vaccination for adult IBD patients treated with immunomodulators and/or anti-TNF-α agents, more effective vaccination methods such as dual vaccinations with booster doses must be established for these patients. Immunogenicity of other types of inactivated vaccines such as the pneumococcal vaccine is also inhibited in IBD patients treated with immunomodulators and/or anti-TNF-α agents.[42] Booster doses of the hepatitis B vaccine, another inactivated vaccine, are recommended.[43] Optimization with a booster dose of the trivalent influenza vaccine is also considered to enhance efficacy in patients with rheumatoid arthritis who are additionally receiving treatment with immunosuppressive drugs.[44,45] We are currently investigating the efficacy of a booster dose of the trivalent influenza vaccine in adult IBD patients undergoing immunosuppressive therapy (UMIN000009259). Furthermore, the development of personalized vaccination plans according to pre-vaccination antibody titer, treatment drugs, and immunologic potential, is expected in the near future.[46]

In conclusion, treatment with infliximab and/or immunomodulators inhibits the immune response to trivalent influenza vaccination in adult IBD patients. These findings should contribute to the development of optimized and personalized influenza vaccines.

Conflict of interest

No conflicts of interest exist.

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References

1. Abraham C, Cho JH. Functional consequences of NOD2(CARD15) mutations. *Inflamm Bowel Dis* 2006;**12**(7):641–50.
2. Aberra FN, Lichtenstein GR. Methods to avoid infections in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2005;**11**(7):685–95.
3. Lichtenstein GR, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Chen DM, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. *Clin Gastroenterol Hepatol* 2006;**4**(5):621–30.
4. Viget N, Vernier-Massouille G, Salmon-Ceron D, Yazdanpanah Y, Colombel JF. Opportunistic infections in patients with inflammatory bowel disease: prevention and diagnosis. *Gut* 2008;**57**(4):549–58.
5. Toruner M, Loftus Jr EV, Harmsen WS, Zinsmeister AR, Orenstein R, Sandborn WJ, et al. Risk factors for opportunistic infections in patients with inflammatory bowel disease. *Gastroenterology* 2008;**134**(4):929–36.
6. Leung VS, Nguyen MT, Bush TM. Disseminated primary varicella after initiation of infliximab for Crohn's disease. *Am J Gastroenterol* 2004;**99**(12):2503–4.
7. Deutsch DE, Olson AD, Kraker S, Dickinson CJ. Overwhelming varicella pneumonia in a patient with Crohn's disease treated with 6-mercaptopurine. *J Pediatr Gastroenterol Nutr* 1995;**20**(3):351–3.
8. Esteve M, Saro C, González-Huix F, Suarez F, Forné M, Viver JM. Chronic hepatitis B reactivation following infliximab therapy in Crohn's disease patients: need for primary prophylaxis. *Gut* 2004;**53**(9):1363–5.
9. Wasan SK, Baker SE, Skolnik PR, Farraye FA. A practical guide to vaccinating the inflammatory bowel disease patient. *Am J Gastroenterol* 2010;**105**(6):1231–8.
10. Melmed GY. Vaccination strategies for patients with inflammatory bowel disease on immunomodulators and biologics. *Inflamm Bowel Dis* 2009;**15**(9):1410–6.
11. Schanzer DL, Langley JM, Tam TWS. Role of influenza and other respiratory viruses in admissions of adults to Canadian hospitals. *Influenza Other Respi Viruses* 2008;**2**(1):1–8.
12. Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003;**289**(2):179–86.
13. Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, et al. Influenza-associated hospitalizations in the United States. *JAMA* 2004;**292**(11):1333–40.
14. Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al. Emergence of a novel swine-origin influenza A(H1N1) virus in humans. *N Engl J Med* 2009;**360**(25):2605–15.
15. Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, et al. Hospitalized patients with 2009 H1N1 influenza in the United States, April–June 2009. *N Engl J Med* 2009;**361**(20):1935–44.
16. deBruyn JC, Hilsden R, Fonseca K, Russell ML, Kaplan GG, Vanderkooi O, et al. Immunogenicity and safety of influenza vaccination in children with bowel disease. *Inflamm Bowel Dis* 2012;**18**(1):25–33.
17. Cullen G, Bader C, Korzenik JR, Sands BE. Serological response to the 2009 H1N1 influenza vaccination in patients with inflammatory bowel disease. *Gut* 2012;**61**(3):385–91.

18. Andrisani G, Frasca D, Romero M, Armuzzi A, Felice C, Marzo M, et al. Immune response to influenza A/H1N1 vaccine in inflammatory bowel disease patients treated with anti TNF-α agents: effects of combined therapy with immunosuppressants. *J Crohns Colitis* 2013;**7**(4):301–7.
19. Kyriakos N, Papamichael K, Archavlis E, Agalos G, Mantzaris GJ. Long term safety and efficacy of H1N1 vaccine in a single-center cohort of IBD patients treated with immunomodulators and/or anti-TNFα biologics. *J Crohns Colitis* 2012;**6**(2):264–5.
20. Rahier JF, Papay P, Salleron J, Sebastian S, Marzo M, Peyrin-Biroulet L, et al. H1N1 vaccines in a large observational cohort of patients with inflammatory bowel disease treated with immunomodulators and biological therapy. *Gut* 2011;**60**(4): 456–62.
21. Fields SW, Baiocco PJ, Korelitz BI. Influenza vaccinations: should they really be encouraged for IBD patients being treated with immunosuppressives? *Inflamm Bowel Dis* 2009;**15**(5):649–51.
22. Mamula P, Markowitz JE, Piccoli DA, Klimov A, Cohen L, Baldassano RN. Immune response to influenza vaccine in pediatric patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2007;**5**(7):851–6.
23. Naganuma M, Fujii T, Kunisaki R, Yoshimura N, Takazoe M, Takeuchi Y, et al. Incidence and characteristics of the 2009 influenza (H1N1) infections in inflammatory bowel disease patients. *J Crohns Colitis* 2013;**7**(4):308–13.
24. Lu Y, Jacobson DL, Ashworth LA, Grand RJ, Meyer AL, McNeal MM, et al. Immune response to influenza vaccine in children with inflammatory bowel disease. *Am J Gastroenterol* 2009;**104**(2): 444–53.
25. European Committee for Proprietary Medical Products. Note for guidance on harmonisation of requirements for influenza vaccines (CPMP/BWP/214/96). London: European Agency for the Evaluation of Medical Products; 1997.
26. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. *BMJ* 1989;**298**(6666):82–6.
27. Best WR, Becktel JM, Singleton JW, Kern Jr F. Development of a Crohn's disease activity index. National cooperative Crohn's disease study. *Gastroenterology* 1976;**70**(3):439–44.
28. Best WR, Becktel JM, Singleton JW. Rederived values of the eight coefficients of the Crohn's disease activity index (CDAI). *Gastroenterology* 1979;**77**(4 Pt 2):843–6.
29. Ohfuji S, Fukushima W, Deguchi M, Kawabata K, Yoshida H, Hatayama H, et al. Immunogenicity of a monovalent 2009 influenza A(H1N1) vaccine among pregnant women: lowered antibody response by prior seasonal vaccination. *J Infect Dis* 2011;**203**(9):1301–8.
30. Hirst GK. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. *J Exp Med* 1942;**75**(1):49–64.
31. Committee for proprietary medicinal products (CPMP). Note for guidance on harmonisation of requirements for influenza vaccines. London: The European Agency for the Evaluation of Medicinal Products; 1997;1–18 [EMEA/CPMP/BWP/214/96].
32. Colombel JF, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010;**362**(15):1383–95.
33. Melmed GY, Ippoliti AF, Papadakis KA, Tran TT, Birt JL, Lee SK, et al. Patients with inflammatory bowel disease are at risk for vaccine-preventable illnesses. *Am J Gastroenterol* 2006;**101**(8): 1834–40.
34. Wasan SK, Coukos JA, Farraye FA. Vaccinating the inflammatory bowel disease patient: deficiencies in gastroenterologist's knowledge. *Inflamm Bowel Dis* 2011;**17**(12):2536–40.
35. Sands BE, Cuffari C, Katz J, Kugathasan S, Onken J, Vitek C, et al. Guidelines for immunizations in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2004;**10**(5):677–92.
36. Narula N, Dhillon AS, Chauhan U, Marshall JK. An audit of influenza vaccination status in adults with inflammatory bowel disease. *Can J Gastroenterol* 2012;**26**(9):593–6.
37. Kunisaki KM, Janoff EN. Influenza in immunosuppressed populations: A review of infection frequency, morbidity, mortality, and vaccine responses. *Lancet Infect Dis* 2009;**9**(8):493–504.
38. Dotan I, Werner L, Vigodman S, Agarwal S, Pfeffer J, Horowitz N, et al. Normal response to vaccines in inflammatory bowel disease patients treated with thiopurines. *Inflamm Bowel Dis* 2012;**18**(2):261–8.
39. França IL, Ribeiro AC, Aikawa NE, Saad CG, Moraes JC, Goldstein-Schainberg C, et al. TNF blockers show distinct patterns of immune response to the pandemic influenza A H1N1 vaccine in inflammatory arthritis patients. *Rheumatology (Oxford)* 2012;**51**(11):2091–8.
40. Romanowska M, Banaszkiewicz A, Nowak I, Radzikowski A, Brydak LB. Immunization against influenza during the 2005/2006 epidemic season and the humoral response in children with diagnosed inflammatory bowel disease (IBD). *Med Sci Monit* 2010;**16**(9):CR433–9.
41. Kobie JJ, Zheng B, Bryk P, Barnes M, Ritchlin CT, Tabechian DA, et al. Decreased influenza-specific B cell responses in rheumatoid arthritis patients treated with anti-tumor necrosis factor. *Arthritis Res Ther* 2011;**13**(6):R209.
42. Fiorino G, Peyrin-Biroulet L, Naccarato P, Szabò H, Sociale OR, Vetrano S, et al. Effects of immunosuppression on immune response to pneumococcal vaccine in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis* 2012;**18**(6): 1042–7.
43. Moses J, Alkhouri N, Shannon A, Raig K, Lopez R, Danziger-lsakov L, et al. Hepatitis B immunity and response to booster vaccination in children with inflammatory bowel disease treated with infliximab. *Am J Gastroenterol* 2012;**107**(1):133–8.
44. Ribeiro AC, Guedes LK, Moraes JC, Saad CG, Aikawa NE, Calich AL, et al. Reduced seroprotection after pandemic H1N1 influenza adjuvant-free vaccination in patients with rheumatoid arthritis: implications for clinical practice. *Ann Rheum Dis* 2011;**70**(12): 2144–7.
45. Saad CG, Borba EF, Aikawa NE, Silva CA, Pereira RM, Calich AL, et al, Timenetsky MDOC, Precioso AR, Bonfa E. Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases. *Ann Rheum Dis* 2011;**70**(6):1068–73.
46. Esteve Comas M, Loras Alastruey C, Fernandez-Bañares F. How do we manage vaccinations in patients with inflammatory bowel disease? *Dig Dis* 2009;**27**(3):370–4.

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Economic evaluation of vaccination programme of mumps vaccine to the birth cohort in Japan

Shu-ling Hoshi*, Masahide Kondo, Ichiro Okubo

Department of Health Care Policy and Health Economics, Faculty of Medicine, University of Tsukuba, 1-1-1, Tennoudai, Tsukuba, Ibaraki 3058577, Japan

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ABSTRACT

The most common preventative measure against mumps is vaccination with mumps vaccine. In most parts of the world, mumps vaccine is routinely delivered through live attenuated Measles-Mumps-Rubella (MMR) vaccine. In Japan, receiving mumps vaccine is voluntary and vaccine uptake rate is less than 30%. The introduction of mumps vaccine into routine vaccination schedule has become one of the current topics in health policy and has raised the need to evaluate efficient ways in protecting children from mumps-related diseases in Japan.

We conducted a cost-effectiveness analysis with Markov model and calculated incremental cost effectiveness ratios (ICERs) of 11 different programmes; a single-dose programme at 12–16 months and 10 two-dose programmes with second dose uptakes at ages 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11. Our base-case analyse set the cost per shot at ¥6951 (US$72; 1US$ = 96.8).

Results show that single-dose programme dominates status quo. On the other hand, ICERs of all 10 two-dose programmes are under ¥6,300,000 (US$65,082) per QALY from payer's perspective while it ranged from cost-saving to <¥7,000,000 (US$72,314) per QALY from societal perspective.

By adopting WHO's classification that an intervention is cost-effective if ICER (in QALY) is between one and three times of GDP as a criterion, either of the vaccination programme is concluded as cost-effective from payer's or societal perspectives. Likewise, to uptake second dose at 3–5 years old is more favourable than an uptake at any other age because of lower incremental cost-effectiveness ratios.

1. Introduction

Mumps is a viral infection of humans, primarily affecting the salivary glands. Serious complications of mumps include meningitis, encephalitis, orchitis, and hearing loss. There is no specific therapy for mumps. In most countries, live attenuated Measles-Mumps-Rubella (MMR) immunisation is delivered against mumps which dropped the incidence of mumps dramatically [1,2]. By December 2005, two-dose schedules were implemented in more than 80% of 110 countries where mumps vaccine is on routine immunisation schedule [1].

In Japan a voluntary mumps vaccination begun in 1981. From 1989, MMR vaccination has been allowed as an alternative to monovalent mumps vaccine for routine immunisation. However, because of unexpected high incidence of aseptic meningitis caused by mumps vaccine (Urabe Am9 strain), MMR vaccination was discontinued in 1993. Since then, measles and rubella vaccines have been in routine vaccination schedule, while mumps monovalent vaccine has been optional as it was before 1989 [3]. Currently, two kinds of mumps vaccine are available in Japan, each containing different strains, namely, Torii and Hoshino [4]. Despite some municipalities giving subsidies to vaccinees to encourage the uptake of mumps vaccine, the estimated vaccine uptake rate is less than 30% [4]. Consequently, Japan has experienced annual outbreaks of mumps estimated from 430,000 to more than one million cases [5], and thus an increase in hearing loss caused by mumps was also observed [6]. The introduction of mumps vaccine into routine vaccination schedule has become one of the current topics in health policy [7] and has raised the need to evaluate efficient ways in protecting children from mumps-related diseases in Japan.

The efficiency of mumps vaccination has been reported overseas since 1970s. Either single-dose strategy or two-dose strategy was shown to be cost-beneficial [8–12]. In Japan, only one peer-reviewed article [13] reported a benefit-cost ratio of 5.1 for single-dose mumps vaccination programme from societal perspective

* Corresponding author. Tel.: +81 29 853 3255; fax: +81 29 853 3255.
E-mail addresses: hoshi@hcs.tsukuba.ac.jp, shulingst@hotmail.com (S.-l. Hoshi).

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with an unrealistic assumption of 100% non-vaccinee infection. The same study also assumed that there is no waning of vaccine-derived immunity, which contradicts the findings of several studies where waning of vaccine-derived immunity is observed [14–21].

This study aims to appraise the value for money of expanding the current voluntary mumps vaccination to routine single-dose or two-dose vaccination programmes, and also to explore the potential impacts of schedule changes, i.e., the appropriate age to uptake the second dose, because of the variety of ages being recommended to uptake the second dose among countries where two-dose MMR is recommended [22,23].

2. Method

We conducted a cost-effectiveness analysis with Markov modelling from both payer's and societal perspectives. In defining vaccination programmes and constructing the model, we conducted a literature survey to find out the available evidence. Studies pertaining to epidemiology and prognosis of mumps-relevant disease in Japan's setting were accessed from PubMed database, Igaku Chuo Zasshi database, MHLW (Ministry of Health, Labour and Welfare) Grant System, and annual statistic reports published by the government. Igaku Chuo Zasshi (Japana Centra Revuo Medicina) is a Japanese medical bibliographic database which contains 7.5 million citations originating in Japan, which comprehensively covers articles published in Japanese-language medical journals. Due to insufficient evidences from Japan, overseas' reports from PubMed, Medline, The Cochrane Database of Systematic Reviews, HTA (Health Technology Assessment database), and NHS EED (The NHS Economic Evaluation Database) regarding vaccine effectiveness, utility weight to estimate QALY and economic evaluation related to mumps vaccine were used instead.

2.1. Programmes

The 11 routine vaccination programmes were composed of one single-dose programme and 10 two-dose programmes. All programmes schedule the first dose at 12–18 months. Each of the 10 two-dose programmes will have the second dose at ages 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11. All these programmes were compared to status quo. We also compared two-dose programmes with single-dose programme to explore the efficiency of the second dose. The vaccine uptake rates are assumed at 30% for status quo [4] and 76% for single-dose programme and for first dose of two-dose programme based on the willingness-to-pay reported by Muta et al. [24], and 72.7% ($76\% \times 0.957$) for second dose of two-dose programme; where 0.957 is the proportion of second dose to first dose of vaccine coverage of measles over the last 5 years in Japan [25]. Vaccination with MMR2 was not considered as an alternative because it is not yet approved in Japan [4,26].

2.2. Markov model

A Markov model of courses followed by the birth cohort under consideration was constructed based on epidemiological data, vaccine effectiveness and models from previous studies. Eleven mutually-exclusive health states were modelled (Fig. 1). A Markov cycle for each stage was set at 1 year with a cohort time frame of 40 years. After turning 40 years old, those without sequelae were assumed to have a life expectancy of Japanese population [27], while those with neurological sequelae will have an average life expectancy of 53.9 years old [28]. Natural infection is thought to confer lifelong protection [1]. Considering that all state transitions do not occur simultaneously at the end of each cycle, we implemented a half-cycle correction in estimating the incremental cost effectiveness ratios (ICERs) of the programmes. We did not consider herd immunity in our model because: (1) the reported basic reproduction number of mumps is largely varied from 4 to 12 [29,30], (2) even when the assumption of vaccine effectiveness is as high as 95% for two doses of vaccine, vaccine coverage of 78.9%, 87.7%, 92.1%, 94.7% are needed to reach herd immunity if the corresponding reproductive values were four, six, eight, and 10; respectively [17], and (3) the experience of unexpected high incidence of aseptic meningitis caused by mumps vaccine in MMR during 1989–1993 in Japan [3] became a barrier to raise vaccine coverage in reaching herd immunity [31].

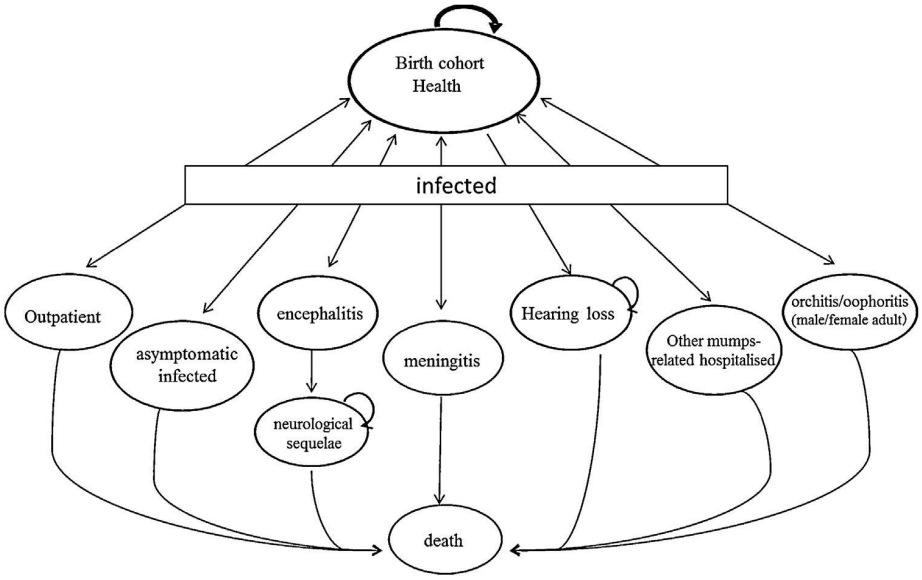

Fig. 1. Markov model. Eleven mutually-exclusive health states were modelled: health, asymptomatic infected, symptomatic infected (outpatient), hospitalised due to meningitis, encephalitis, neurological sequelae due to encephalitis, hearing loss, other mumps-related hospitalisation (including pancreatitis, myocarditis, severe mumps without complication), hospitalised due to orchitis/oophoritis (male/female adult patient only), and death of or other than the related diseases.

2.3. Outcomes estimation

Outcomes in terms of quality adjusted life year (QALY) were estimated by assigning transition probabilities and utility weights from literature to the Markov model.

Age-specific annual incidence rates of symptomatic mumps case were estimated by combining data from three reports: (1) a study grant funded by the Ministry of Health, Welfare and Labor, which estimated the nationwide mumps cases from 2000 through 2007 based on sentinel surveillance reports [5], (2) age distribution of mumps cases reported by Infectious Disease Surveillance Center [32], and (3) population data [33]. As to the incidence rates of asymptomatic mumps case, previous studies reported that approximately 15–40% of mumps infection is subclinical and the younger the age, the higher is the proportion of subclinical to symptomatic infection [1,34]. We assumed that the proportion is linearly decreased from 40% for those aged <2 to 15% for aged 20 to <40. These data are shown in Table 1.

Proportion of hearing loss among symptomatic mumps cases, 1 in 1000 cases, is from a prospective study which enrolled 7502 mumps patients from 40 institutes in Japan [35]. Proportion of meningitis, encephalitis, orchitis, oophoritis, and other mumps-related hospitalisation cases were estimated by using proportion of hearing loss and numbers of relevant disease cases reported by nationwide survey conducted from December 2011 to March 2012 [36]. Proportion that resulted in neurological sequelae among cases of encephalitis age <20 is from the same report [36]. Deaths of causes other than the above diseases were taken from the vital statistics [37]. All these data are shown in Table 2.

Table 1
Estimation of incidences of symptomatic and asymptomatic cases.

(1) Cases of symptomatic mumps estimated by Nagai et al. [5]

2000	1,170,000
2001	2,260,000
2002	1,089,000
2003	515,000
2004	821,000
2005	1,356,000
2006	1,186,000
2007	431,000

(2) Age distribution of symptomatic mumps cases reported by NIID [32]; %

Year	Age <2	Age 2 to <4	Age 4 to <6	Age 6 to <8	Age 8 to <10	Age 10 to <15	Age 15 to <20	Age 20 to <40
2000	5.0	22.3	36.2	20.7	8.1	5.4	0.5	1.8
2001	5.2	23.5	34.9	20.8	8.0	5.3	0.5	1.8
2002	5.1	23.0	34.9	20.3	8.7	5.7	0.6	1.7
2003	4.9	22.1	35.9	20.0	8.9	5.9	0.6	1.8
2004	5.1	24.0	36.4	19.8	7.9	4.7	0.5	1.7
2005	5.2	24.4	35.6	20.0	7.7	4.9	0.5	1.7
2006	5.0	22.6	35.0	20.3	9.1	5.7	0.5	1.8
2007	5.1	22.3	34.4	20.4	9.5	6.3	0.5	1.6

(3) Case of symptomatic mumps estimated from (1) and (2)

Year	Age <2	Age 2 to <4	Age 4 to <6	Age 6 to <8	Age 8 to <10	Age 10 to <15	Age 15 to <20	Age 20 to <40
2000	58,500	260,910	423,540	242,190	94,770	63,180	5,850	21,060
2001	117,520	531,100	788,740	470,080	180,800	119,780	11,300	40,680
2002	55,539	250,470	380,061	221,067	94,743	62,073	6,534	18,513
2003	25,235	113,815	184,885	103,000	45,835	30,385	3,090	9,270
2004	41,871	197,040	298,844	162,558	64,859	38,587	4,105	13,957
2005	70,512	330,864	482,736	271,200	104,412	66,444	6,780	23,052
2006	59,300	268,036	415,100	240,758	107,926	67,602	5,930	21,348
2007	21,981	96,113	148,264	87,924	40,945	27,153	2,155	6,896

(4) Populations [33]

Year	Age <2	Age 2 to <4	Age 4 to <6	Age 6 to <8	Age 8 to <10	Age 10 to <15	Age 15 to <20	Age 20 to <40
2000	2,342,000	2,385,000	2,393,000	2,401,000	2,425,000	6,559,000	7,502,000	35,172,000
2001	2,345,000	2,364,000	2,379,000	2,413,000	2,401,000	6,382,000	7,350,000	35,245,000
2002	2,339,000	2,338,000	2,390,000	2,391,000	2,400,000	6,245,000	7,194,000	35,195,000
2003	2,292,000	2,337,000	2,368,000	2,375,000	2,414,000	6,120,000	6,997,000	35,133,000
2004	2,241,000	2,328,000	2,335,000	2,382,000	2,388,000	6,060,000	6,762,000	34,960,000
2005	2,156,000	2,274,000	2,356,000	2,382,000	2,381,000	6,037,000	6,592,000	34,263,000
2006	2,138,000	2,213,000	2,320,000	2,366,000	2,390,000	6,008,000	6,424,000	34,243,000
2007	2,171,000	2,145,000	2,269,000	2,347,000	2,378,000	5,983,000	6,281,000	33,823,000

(5) Incidence of symptomatic mumps cases per 100,000 population (estimated from (3) and (4))

Aged	<2	2 to <4	4 to <6	6 to <8	8 to <10	10 to <15	15 to <20	20 to <40
	2499.2	11142.0	16598.5	9438.9	3829.0	962.1	83.0	55.7

(6) Incidence of asymptomatic mumps cases per 100,000 population*

Aged	<2	2 to <4	4 to <6	6 to <8	8 to <10	10 to <15	15 to <20	20 to <40
	1666.1	6384.8	8122.6	3909.1	1325.4	273.6	18.9	9.8

* The proportion of subclinical infection cases is assumed linearly decrease from 40% for age <2 to 15% for age 20 to <40 [1,34].

Table 2
Variables.

Variable	Base-case	Value applied on one-way sensitivity analyses		Reference
Vaccine uptake rate		Lower limit	Upper limit	
Status quo	30.0%	–	–	[4]
Single-dose programme	76.0%	–	–	[24]
Two-dose immunisation programme	1st dose: 76.0%	–	–	[24,25]
	2nd dose: 72.7%	–	–	
Annual incidence rates per 100,000 population				
Symptomatic mumps case	Shown on Table 1	−50%	+50%	See Table 1
Symptomatic mumps	Shown on Table 1	−50%	+50%	See Table 1
Proportion of revalent mumps diseases among symptomatic mumps cases				
Healing loss	0.10%	0.05%	0.15%	[35]
Meningitis	2.23%	1.12%	3.35%	[36]
Encephalitis	0.05%	0.02%	0.07%	[36]
Orchitis (male, ≥20 years old)	25.00%	12.50%	37.50%	[36]
Oophoritis (female, ≥20 years old)	5.00%	2.50%	7.50%	[36]
Other mumpus-related hospitalization	1.52%	0.76%	2.27%	[36]
Outpatient	66.11%	33.05%	99.16%	[36]
Proportion of encephalitis cases under 20 years old resulted in neurological sequelae	0.43%	0.21%	0.64%	[36]
Proportion of hearing loss cases resulted in bilateral hearing loss	2.00%	1.00%	3.00%	Assumed
Vaccine effectiveness in reducing symptomatic cases				
First-dose	69.6%	54.0%	87.0%	[14,17–20,38]
Second-dose	87.0%	69.6%	93.0%	[14,17–20,38]
Waning of vaccine-derived immunity	75% in 20 years	50% in 20 years	–	[15]
Life expectancy of Japanese population at age 40/year	41.05 male; 47.17 female			[27]
Life expectancy of neurological sequelae at age 40/year	13.9	–	–	[28]
Utility weight				[12,13,28]
Healthy,	1	–	–	
Hearing loss, unilateral	0.900	0.720	1	
Hearing loss, bilateral	0.800	0.640	0.900	
Neurological sequelae	0.570	0.456	0.684	
Curable encephalitis	0.977	0.781	1	
Curable meningitis	0.977	0.781	1	
Hospitalisation other than above diseases	0.990	0.792	–	
Death	0	–	–	
Cost				
Cost per shot	¥6972	¥3486	¥10,458	[40]
Treatment cost per case				
Meningitis/Encephalitis episode	¥852,642	¥426,321	¥1,278,963	[41]
Unilateral hearing loss	¥79,422	¥39,711	¥119,133	[42]
Bilateral hearing loss	¥4,000,000	¥2,000,000	¥6,000,000	[44]
Orchitis	¥171,732	¥85,866	¥257,598	[43]
Oophoritis	¥186,905	¥93,453	¥280,358	[43]
Hospitalised due to other than the above complications	¥233,200	¥116,600	¥349,800	[13]
Outpatient	¥10,477	¥5239	¥15,716	[13]
Neurological sequelae (long-term trearment cost per case per year)	¥420,464	¥210,232	¥630,696	[41]
Discount rate	3.0%	0%	5.0%	[39]
Variables related to care-giver's productivity loss				
Uptake vaccine	4 h, if uptake alone; zero, if co-vaccinated with other vaccine			
Meningitis/Encephalitis episode	22.7 days			[41]
Unilateral hearing impairment	8 h per day until the child is admitted to special support education system			
Bilateral hearing impairment				
Neurological sequelae				
Orchitis	4.9 days			[43]
Oophoritis	5.3 days			[43]
Other mumps-related hospitalisation	5 days			[13]
Outpatient	5 days (schooldays suspension)			[13]
Average hourly wage of Japanese women labourers	¥1328			[45]

Case-fatality rate of encephalitis.

2.4. Vaccine effectiveness and waning of vaccine-derived immunity

Due to low uptake of mumps vaccine, data regarding vaccine effectiveness or efficacy are scarce in Japan. After reviewing researches from overseas [14,17–20,38], we assumed that vaccine effectiveness in reducing infection is 69.6% for the first dose and 80% for the second dose. Kontio et al.'s [15] findings which regarded that waning of vaccine-derived immunity will decrease by 75% in 20 years was also used in the study, and from which we assumed the remaining 25% to last until the end of the model.

2.5. Costing

From societal perspective, costing should cover opportunity costs borne by various economic entities in society [39]. Therefore,

costs of vaccination, treatment costs of mumps-related diseases, and costs associated to care-giver's productivity loss, were counted. Productivity loss due to mortality was not included because it can be argued as double counting, while survived cases were incorporated in utility weights and disease duration in calculating QALYs [39]. From the payer's perspective, care-giver's productivity loss was not included. All variables related to costs are shown in Table 2.

2.5.1. Direct medical costs

Vaccination cost per shot was assumed at ¥6951 (US$72; 1US$ = ¥96.8, average of 2013) [40], which was estimated as sum of the following: (1) doctor's fee for medical advice (¥3450, US$35.6), (2) technical fee for administering vaccine (¥330, US$30.4), (3) price of vaccine (¥2840, US$29.3) and (4) tax [40]. The doctor's fee and technical fee are from the National fee schedule, while the vaccine price comes from the average company prices of mumps vaccine in Japan. Vaccine price is more expensive than those of overseas' due to the vaccine protection and delivery system under strict governmental plan in Japan.

We used Iwata et al.'s treatment cost, ¥852,642 (US$8808) per episode of meningitis, and assumed it to hold through for encephalitis [41]. Likewise, we used Yamanaka et al.'s treatment cost ¥79,422 (US$821) per case of unilateral hearing loss [42]. Orchitis and oophoritis with rates ¥171,732 (US$1774) and ¥186,905 (US$1931) per case, respectively, were based from the Survey on Medical Benefits [43]. For bilateral hearing loss, it was at ¥4,000,000 (US$41,322) per case (including cost of cochlea implant) [44]. We used Sugawara et al.'s [13] treatment cost for cases other than the above diseases and per mumps outpatient at ¥233,000 (US$2407) and ¥10,477 (US$108); respectively. We used Iwata et al.'s [41] estimate for long-term treatment cost for an individual suffering from neurological sequelae at ¥400,000 (US$4132) per year.

2.5.2. Productivity loss

Productivity loss of a care-giver accompanying a child for vaccine uptake was estimated depending on how mumps vaccine was taken. If mumps vaccine was simultaneously taken with any other vaccines already on the routine schedule (i.e. co-vaccinated scenario), no productivity loss will occur. If it was taken alone (i.e. vaccine alone scenario), then productivity loss will be calculated by 4 h × wage of care-giver. Productivity loss per disease episode is valued as a product of care-giver's or patient's absent working hours from paid employment and an average hourly wage that depends on the age of the individual who suffers from the diseases. If the patients are less than 18 years old an average hourly wage of ¥1326 (US$14) for Japanese women workers will be used; otherwise, an age-specific average hourly wage will ensue [45]. For outpatients younger than 18 years old, five school days suspension was assumed. We assumed that a care-giver's absent working hours of taking care of one child with neurological sequelae or hearing impairment is 8 h per day until the child is admitted to special support education system, which is at age 6 in Japan.

2.6. Discounting

Costs and outcomes were discounted at a rate of 3% [39].

3. Sensitivity analyses

We performed one-way sensitivity analyses to appraise the stability of ICERs against assumptions made in our economic model, and to explore the impact of each variable relative to each other. The lower limits and upper limits used on sensitivity analyses are shown in Table 2.

4. Results

4.1. Results of cost-effectiveness analyses

In our base-case analysis, with a comparison to status quo, the estimated mumps cases averted per 100,000 population by the start of routine vaccination programmes followed for 40 years was at 15,206 cases for single-dose programme and from 16,169 cases (uptake second dose at age 11) to 24,734 cases (uptake second dose at age 3) for two-dose programmes.

Table 3 and Fig. 2 show the estimated incremental effects per child ranging from 0.00053 QALY to 0.00086 QALY. Among all the programmes, the two-dose programme with second dose uptake at 3 years old gained the most. All vaccination programmes reduced disease treatment costs. However, except single-dose programme, these reduced costs did not offset vaccination cost, which means the single-dose programme gained more QALY with less cost, while the two-dose programmes turned out to yield more QALY but cost more from payer's perspective. Estimated ICERs of two-dose programmes ranged from ¥2,977,695 (US$30,761) per QALY to ¥6,288,633 (US$64,965) per QALY. Among the two-dose programmes, the lowest ICER was recorded in the second dose uptake at age 4 followed by ages 3, 2, 5, 6, 7, 8, 9, 10, and 11.

In societal perspective, wherein a care-giver's productivity loss was included, the sum of reduced productivity loss due to disease and reduced disease treatment costs offset the sum of vaccination cost and productivity loss due to vaccine uptake, which means these programmes turned out to be cost-saving in single-dose programme and some of two-dose programmes, such as: uptake in alone/co-vaccinated scenario with second dose uptakes at ages 2, 3, 4, 5, 6, and 7. ICERs of programmes which did not turned out to be cost-saving ranged from ¥1,050,933 (US$10,857) per QALY to ¥6,926,263 (US$71,552) per QALY in alone/alone scenarios and ¥1,028,707 (US$10,627) per QALY to ¥1,801,783 (US$18,613) per QALY in alone/co-vaccinated scenarios.

When comparing two-dose programmes with the single-dose programme, ICERS per QALY from payer's perspective ranged from ¥7,997,190 (US$82,616) to ¥122,934,023 (US$1,269,980), while in societal perspective it ranged from ¥9,838,812 (US$101,641) to ¥212,586,977 (US$2,196,146) in alone/alone scenario and from cost-saving to ¥113,454,799 (US$1,172,054) in alone/co-vaccinated scenario.

4.2. Stability of ICER

Fig. 3 shows the top five variables that produced large ICER variations when compared with status quo from payer's perspective. Largest change was seen in costs per shot of vaccine in all programmes. When cost is decreased to half of its base-case, all programmes turned out to have negative ICERs, which means that the implementation of any of these programmes will result in gaining more QALYs with lesser cost. The next top four variables that produced large changes in ICER are any four of the six variables: treatment costs per meningitis case, proportion of meningitis among symptomatic mumps cases, incidence of symptomatic mumps cases, vaccine effectiveness of first dose, vaccine effectiveness of second dose, and utility weight of unilateral hearing loss, whose order are influenced by the programme and age. Among 726 ICERs estimated (66 changes in variables, 11 programmes), 22 ICERs from five variables in two-dose programmes were found to be larger than ¥10,000,000 (US$103,306) per QALY. These resulted because of the: (1) lower limit of incidence of symptomatic mumps cases, if second dose uptake is at age ≥6, (2) lower limit of vaccine effectiveness of the first-dose, if uptake is at age ≥8, (3) upper limit of costs per vaccine shot, if uptake is at ≥7, (4) proportion of meningitis among symptomatic mumps cases, if uptake is at ≥10, and (5)

Table 3
Results of base-case analysis from payer's perspective.

Per child						Cost(¥)/QALY	
Programme (age of 2nd dose)	Vaccine cost	Diseases treatment costs	Total costs	QALY	Incremental effects (QALY)	Compared with status quo	Compared with single-dose programme
Status quo	2085	14,422	16,507	30.77595	–	–	–
1-dose	5283	11,188	16,470	30.77648	0.00053	gained more, cost less	–
2-dose (at 2 yr)	10,191	9182	19,373	30.77680	0.00085	3,370,669	9,126,515
2-dose (at 3 yr)	10,048	9108	19,156	30.77681	0.00086	3,076,136	8,163,270
2-dose (at 4 yr)	9910	9144	19,053	30.77681	0.00085	2,977,695	7,997,190
2-dose (at 5 yr)	9774	9540	19,315	30.77674	0.00079	3,544,574	10,940,253
2-dose (at 6 yr)	9644	10,063	19,707	30.77666	0.00071	4,512,419	18,285,133
2-dose (at 7 yr)	9517	10,348	19,865	30.77661	0.00066	5,064,338	25,914,654
2-dose (at 8 yr)	9394	10,692	20,086	30.77656	0.00061	5,875,233	46,950,392
2-dose (at 9 yr)	9274	10,818	20,091	30.77654	0.00059	6,084,222	63,522,279
2-dose (at 10 yr)	9157	10,909	20,066	30.77652	0.00057	6,199,236	85,607,727
2-dose (at 11 yr)	9045	10,991	20,035	30.77651	0.00056	6,288,633	122,934,023

Results of base-case analysis from societal perspective by

						Cost(¥)/QALY			
	Productivity loss (uptake vaccine) by scenario of uptake 1st-/2nd-vaccine		Productivity loss (diseases treatment)	Total costs		Compared with status quo		Compared with single-dose programme	
	Alone/alone	Alone/ co-vaccinated		Alone/alone	Alone/ co-vaccinated	Alone/alone	Alone/ co-vaccinated	Alone/alone	Alone/ co-vaccinated
Status quo	1594	1594	20,772	38,873	38,873				
1-dose	4037	4037	16,086	36,593	36,593	gained more, cost less	gained more, cost less		
2-dose (at 2 yr)	7788	4037	13,191	40,352	36,601	1,740,071	gained more, cost less	11,819,023	23,460
2-dose (at 3 yr)	7679	4037	13,090	39,925	36,284	1,222,499	gained more, cost less	10,127,539	gained more, cost less
2-dose (at 4 yr)	7573	4037	13,145	39,771	36,236	1,050,933	gained more, cost less	9,838,812	gained more, cost less
2-dose (at 5 yr)	7470	4037	13,716	40,500	37,068	2,055,068	gained more, cost less	15,026,933	1,824,386
2-dose (at 6 yr)	7370	4037	14,469	41,546	38,213	3,769,764	gained more, cost less	27,978,257	9,149,198
2-dose (at 7 yr)	7273	4037	14,881	42,019	38,783	4,744,663	gained more, cost less	41,412,979	16,713,355
2-dose (at 8 yr)	7179	4037	15,377	42,641	39,499	6,186,973	1,028,707	78,535,788	37,738,593
2-dose (at 9 yr)	7087	4037	15,559	42,737	39,687	6,560,788	1,382,617	107,784,091	54,276,315
2-dose (at 10 yr)	6998	4037	15,691	42,755	39,794	6,764,205	1,605,623	146,715,293	76,214,681
2-dose (at 11 yr)	6912	4037	15,811	42,758	39,884	6,926,263	1,801,783	212,586,977	113,454,799

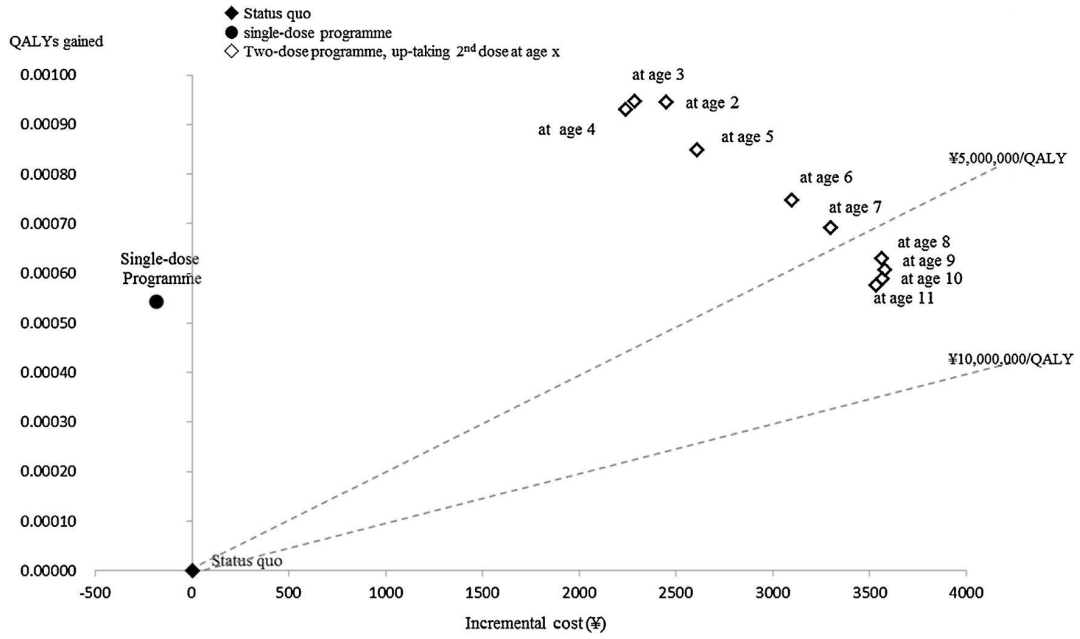

Fig. 2. Cost-effectiveness plane.

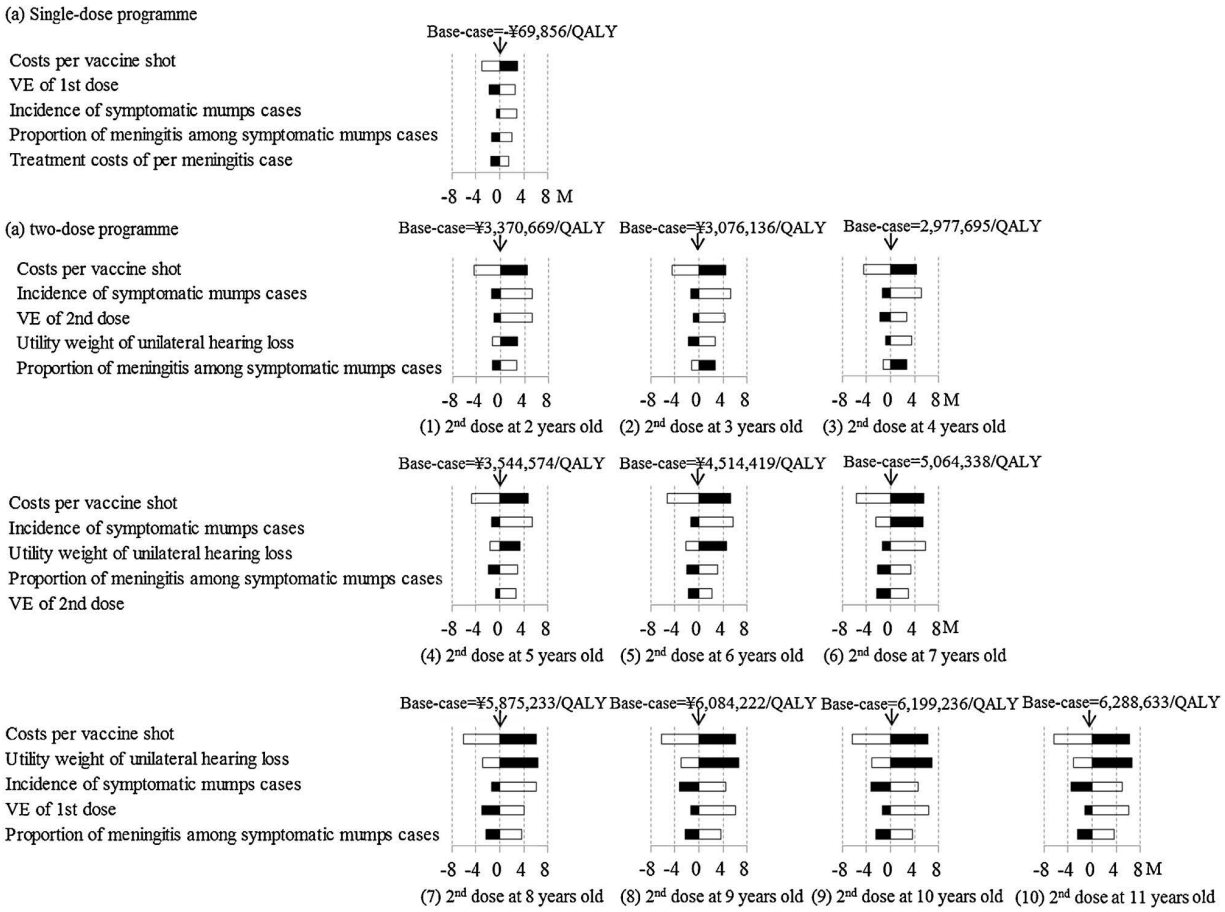

Fig. 3. Results of one-way sensitivity analysis. Variables were changed one at a time when performing one-way sensitivity analysis.

upper limit of utility weight of unilateral hearing loss, if uptake is at age ≥7.

5. Discussion

We conducted cost-effectiveness analyses on routine mumps vaccine immunisation programmes for the birth cohort in Japan. There were 11 different programmes, a single-dose programme at 12–16 months and 10 two-dose programmes with second dose uptakes at ages 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11. Analyses were done from both societal (with productivity loss) and payer's perspectives (without productivity loss).

The single-dose programme gained more QALY with less cost when compared with status quo. ICERs of all 10 two-dose programmes are under ¥6,300,000 (US$65,083) per QALY from payer's perspective; while it ranged from cost-saving to <¥7,000,000 (US$72,314) per QALY from societal perspective. A willingness-to-pay threshold, ¥5,000,000 (US$51,653) per QALY gained, has been suggested for healthcare intervention [46], while WHO suggests three times of GDP (around ¥11,000,000 or US$113,636 in Japan) as a criterion to judge whether an immunisation programme is cost-effective or not [47]. By using the ¥10,000,000 (US$103,306) threshold, all programmes in our model can be concluded as cost-effective. Moreover, the single-dose programme is deemed to be cost-saving regardless of the perspective. Among the 10 two-dose programmes, second dose uptake at 3 or 4 years old has lower ICER than others, also these two programmes turned out to be cost-saving from societal perspective when vaccine uptake was done simultaneously with other vaccine. When compared with the single-dose programme, ICERs of additional second dose will be lower than ¥10,000,000 (US$103,306) per QALY if the second dose of the vaccine uptake is ≤4 years old from payer's perspective, at 4 years old from societal perspective in the vaccine alone scenario, and if ≤6 years old in co-vaccinated scenario. Comparing ICERs of the programmes with PCV-7, about ¥7,400,000 per QALY [27], which is now on the list of routine immunisation schedule in Japan, the two-dose mumps vaccination programme are considered to be more favourable. With these results, when routine mumps vaccination programme were to be implemented, two-dose programme with second dose schedule at 3–5 years old are favourable than schedules at higher ages.

Our conclusions are considered robust based on the results from our sensitivity analyses: only 22 out of 726 ICERs exceeded ¥10,000,000 per QALY and the largest ICER is less than ¥13,200,000 (US$136,363) per QALY. Also, five out of these 22 ICERs are from the upper limit at 150% base-case cost of cost per vaccine shot. Cost per shot rising to 150% from current costs is relatively low because of the strict vaccine protection and delivery system in Japan.

Studies from overseas reported that single- or two-dose mumps vaccination programmes as highly cost-beneficial, in which mumps vaccine was given through measles-mumps-rubella combination [8–12]. In Japan, only one peer-reviewed article reported an incremental benefit-cost ratio of 5.1 for single-dose mumps vaccination programme only from societal perspective and unrealistically assumed that 100% of non-vaccinees will be infected [13], which we consider as an over evaluation due to improbable assumptions. Though it is difficult to directly compare the results of economic evaluation among different countries or even within a country due to model and parameter variations, our analysis from societal perspective shows that single-dose and two-dose programmes with second dose uptake at 2–7 years old were cost-saving, which is consistent with the results of previous studies.

This study has limitations. First, clinical evidence of the efficiency of vaccination in reducing annual incidence rates of mumps cases in the model were adopted from studies carried out in other countries since no similar study has been done in Japan. There should be differences in vaccine strains, in ethnicity, as well as in healthcare system between those countries and Japan. Second, proportion of meningitis, encephalitis, orchitis, oophoritis, and other mumps-related hospitalisation cases among symptomatic mumps cases were indirectly estimated by using a nationwide survey jointly conducted by Japan Medical Association, Japan Pediatric Association, and Japan Pediatric Society [36] wherein the response rate of the survey is not high enough to ensure against bias. Third, though aseptic meningitis is a side effect of mumps vaccine, we did not include it in our model. A study, which enrolled 1051 children with mumps and 21,465 vaccine recipients by 143 paediatric primary care practitioners from 2000 to 2003, reported an incidence of aseptic meningitis at 1.24% in patients with symptomatic natural mumps infection and 0.05% in vaccine recipients [34]; hence, its inclusion would bring more favourable results to the vaccination programmes.

Regardless of these limitations, our model considers the potential impact of waning immunity and schedule changes, i.e. different ages of up-taking the second dose, which is unique in the economic evaluation of mumps vaccine in terms of context of choice under consideration.

6. Conclusion

A routine vaccination programme of single-dose is cost-saving from both payer's and societal perspectives. All two-dose programmes are considered cost-effective from both perspectives. Among them, second dose uptake at age 3, 4 and 5 are recommended because they are highly cost-effective from payer's perspective and will turn out to be cost-saving from societal perspective.

Author's contributions

Shu-Ling Hoshi participated in the concept and design of the study, performed the literature searches, acquired the data, participated in the analysis and interpretation of the data, and wrote the manuscript. Masahide Kondo and Ichiro Okubo participated in the concept and design of the study, the interpretation of the data.

Sponsors role

None.

Conflict of interest

None.

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References

[1] WHO position paper on mumps virus vaccines. Weekly Epidemiological Record No 7. 16 February 2007.
[2] Peltola H, Davidkin I, Paunio M, Valle M, Leinikki P, Heinonen OP. Mumps and rubella eliminated from Finland. JAMA 2000;284(20):2643–7.
[3] Nakayama T. Vaccine chronicle in Japan. J Infect Chemother 2013;19:787–98.
[4] Infectious agents surveillance report (IASR). Mumps 1993~2002, 24. IASR; 2003. p. 103–4 [in Japanese].

[5] Nagai M, Hashimoto S, Kawado M, Taniguchi K, Shigematu M, Tada Y, et al. Kansensho haseidoukou tyousa ni motozuku ryuko no keiho chuiho oyobi zenkoku nenkan rikansu no suikei. Report of health and labour sciences research grants. Tokyo: Ministry of Health, Labour, and Welfare; 2008. p. 129–34 [In Japanese].
[6] Kawashima Y, Ihara K, Nakamura M, Nakashima T, Fukuda S, Kitamura K. Epidemiological study of mumps deafness in Japan. Auris Nasus Larynx 2005;32(2):125–8.
[7] Sasaki T, Tsunoda K. Time to revisit mumps vaccination in Japan? Lancet 2009;374(9702):1722.
[8] Wiedermann G, Ambrosch F. Cost-benefit calculations of vaccinations against measles and mumps in Austria. Dev Biol Stand 1979;43:273–7.
[9] Koplan JP, Preblud SR. A benefit-cost analysis of mumps vaccine. Am J Dis Child 1982;136(4):362–4.
[10] White CC, Koplan JP, Orenstein WA. Benefits, risks and costs of immunization for measles, mumps and rubella. Am J Public Health 1985;75(7):739–44.
[11] Rivière M, Tretiak R, Levinton C, Fitzsimon C, Leclerc C. Economic benefits of a routine second dose of combined measles, mumps and rubella vaccine in Canada. Can J Infect Dis 1997;8(5):257–64.
[12] Zhou F, Reef S, Massoudi M, Papania MJ, Yusuf HR, Bardenheier B, et al. An economic analysis of the current universal two-dose measles-mumps-rubella vaccination program in the United States. J Infect Dis 2004;189:S131–45.
[13] Sugawara T, Ohkusa Y, Taya K, Oikawa K, Haneda N, Kikuchi K, et al. Cost-effectiveness analysis of routine mumps immunization in Japan. Kansenshogaku Zasshi 2007;81(5):555–61 [in Japanese].
[14] Dayan GH, Rubin S. Mumps outbreaks in vaccinated populations: are available mumps vaccines effective enough to prevent outbreaks? Clin Infect Dis 2008;47(11):1458–67.
[15] Kontio M, Jokinen S, Paunio M, Peltola H, Davidkin I. Waning antibody levels and avidity: implications for MMR vaccine-induced protection. J Infect Dis 2012;206(10):1542–8.
[16] Castilla J, García Cenoz M, Arriazu M, Fernández-Alonso M, Martínez-Artola V, Etxeberria J, et al. Effectiveness of Jeryl Lynn-containing vaccine in Spanish children. Vaccine 2009;27(15):2089–93.
[17] Deeks SL, Lim GH, Simpson MA, Gagné L, Gubbay J, Kristjanson E, et al. An assessment of mumps vaccine effectiveness by dose during an outbreak in Canada. CMAJ 2011;183(9):1014–20.
[18] Centers for Disease Control and Prevention. Prevention of measles, rubella, congenital rubella syndrome, and mumps, 2013: summary recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2013;62(RR-04):1–34.
[19] Poethko-Müller C, Mankertz A. Seroprevalence of measles-, mumps- and rubella-specific IgG antibodies in German children and adolescents and predictors for seronegativity. PLoS One 2012;7(8):e42867.
[20] Peltola H, Kulkarni PS, Kapre SV, Paunio M, Jadhav SS, Dhere RM. Mumps outbreaks in Canada and the United States: time for new thinking on mumps vaccin. Clin Infect Dis 2007;45(4):459–66.
[21] Davidkin I, Jokinen S, Broman M, Leinikki P, Peltola H. Persistence of measles, mumps, and rubella antibodies in an MMR-vaccinated cohort: a 20-year follow-up. J Infect Dis 2008;197(7):950–6.
[22] Wood JG, Gidding HF, Heywood A, Macartney K, McIntyre PB, Macintyre CR. Potential impacts of schedule changes, waning immunity and vaccine uptake on measles elimination in Australia. Vaccine 2009;27(2):313–8.
[23] American Academy of Pediatrics. Committee on infectious diseases. Age for routine administration of the second dose of Measles-Mumps-Rubella vaccine. Pediatrics 1998;101:129–33.
[24] Muta H, Oshiumi K, Matsuda Y. Cost of vaccination and parents' willingness to vaccinate: comparson of Haemophilus influenza type b and mumps vaccines. J Ambul Gen Pediatr 2012;15(1):2–12 [in Japanese].
[25] Ministry of Health, Labour and Welfare [in Japanese] http://www.mhlw.go.jp/topics/bcg/other/5.html
[26] Murashige N. Japan's immunisation policy in routine, pandemic and post-tsunami situations. Int J Clin Pract 2011;65(11):1126–31.
[27] Ministry of Health, Labour and Welfare. The 20th life tables. Tokyo: Health and Welfare Statistics Association; 2007 [in Japanese].
[28] Hoshi SL, Kondo M, Okubo I. Economic evaluation of vaccination programme of 7-valent pneumococcal conjugate vaccine to the birth cohort in Japan. Vaccine 2012;30(22):3320–8.
[29] Anderson RM, May RM. Immunisation and herd immunity. Lancet 1990;335(8690(Mar)):641–5.
[30] Fine PE. Herd immunity: history, theory, practice. Epidemiol Rev 1993;15(2):265–302.
[31] Baba K, Okuno Y, Tanaka-Taya K, Okabe N. Immunization coverage and natural infection rates of vaccine-preventable diseases among children by questionnaire survey in 2005 in Japan. Vaccine 2011;29(16):3089–92.
[32] Infectious Disease Surveillance Center. Infectious Diseases Weekly Report Japan (IDWR) 2010;12(19):13–5 [in Japanese].
[33] Ministry of Internal Affairs and Communications. Population estimates of Japan. Tokyo: Statistics Bureau; 2011 [In Japanese].
[34] Nagai T, Okafuji T, Miyazaki C, Ito Y, Kamada M, Kumagai T, et al. A comparative study of the incidence of aseptic meningitis in symptomatic natural mumps patients and monovalent mumps vaccine recipients in Japan. Vaccine 2007;25(14):2742–7.
[35] Hashimoto H, Fujioka M, Kinumaki H, Kinki Ambulatory Pediatrics Study Group. An office-based prospective study of deafness in mumps. Pediatr Infect Dis J 2009;28(3):173–5.
[36] Hosaka S, Komori K, Hoshina K, Mine M, Hosoya M, Igarashi T, et al. Investigation about serious cases caused by mumps virus and varicella-zoster virus. J Jpn Pediatr Assoc 2012;44:182–6 [in Japanese].
[37] Ministry of Health, Labour and Welfare. Vital statistics of Japan 2012. Tokyo: Health and Welfare Statistics Association; 2013 [in Japanese].
[38] Demicheli V, Rivetti A, Debalini MG, Di Pietrantonj C. Vaccines for measles, mumps and rubella in children. Cochrane Database Syst Rev 2012;(2), http://dx.doi.org/10.1002/14651858.CD004407.pub3. Art. No.: CD004407.
[39] Drummond MF, Sculpher MJ, Torrance GW, O'Brien BJ, Stoddart GL. Methods for economic evaluation of health care programmes. 3rd ed. Oxford: Oxford University Press; 2004.
[40] The subcommittee about vaccine evaluation. Otahuku kaze wakutin sagyou timu houkokusyo [Mumps vaccine work team report]. Tokyo: Ministry of Health, Labour and Welfare relation Council; 2011 [in Japanese].
[41] Iwata S, Ishiwada N, Sakata H, Sakano T, Sato Y, Nakano T, et al. Burden of illness of bacterial meningitis and bacteremia caused by Streptococcus pneumonia in children. Jpn J Pediatr 2008;61:2206–20 [in Japanese].
[42] Yamanaka N, Hotomi M, Sugita R. Cost-effectiveness of pneumococcal conjugate vaccine in Japan: estimation on acute pneumococcal otitis media in children. Jpn J Pediatr 2008;61:2221–32 [in Japanese].
[43] Medical Economics Division, Health Insurance Bureau. Iryou kyuhu jitai tyousa. Tokyou: Ministry of Health, Labour and Welfare; 2010 [In Japanese].
[44] Okubo S, Takahashi M, Kai I. How Japanese parents of deaf children arrive at decisions regarding pediatric cochlear implantation surgery: a qualitative study. Soc Sci Med 2008;66(12):2436–47.
[45] Ministry of Health, Labour and Welfare. Basic survey on wage structure 2009. Tokyo: Health and Welfare Statistics Association; 2010 [in Japanese].
[46] Shiroiwa T, Sung YK, Fukuda T, Lang HC, Bae SC, Tsutani K. International survey on willingness-to-pay (WTP) for one additional QALY gained: what is the threshold of cost effectiveness? Health Econ 2010;19(4):422–37.
[47] World Health Organization. WHO guide for standardization of economic evaluations of immunization programmes. Geneva, Switzerland: WHO Document Production Services; 2008.

RESEARCH PAPER

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Immunogenicity of a monovalent influenza A(H1N1)pdm09 vaccine in patients with hematological malignancies

Yuichiro Ide[1,*], Yutaka Imamura[2], Satoko Ohfuji[1], Wakaba Fukushima[1], Saburo Ide[3], Chiyo Tsutsumi[3], Masahisa Koga[4], Kazuhiro Maeda[5], and Yoshio Hirota[1]

[1]Department of Public Health; Osaka City University Graduate School of Medicine; Osaka, Japan; [2]Department of Hematology; St. Mary's hospital; Fukuoka, Japan; [3]Faculty of Nursing; St. Mary's College; Fukuoka, Japan; [4]Department of Clinical Laboratory; St. Mary's Hospital; Fukuoka, Japan; [5]The Research Foundation for Microbial Disease of Osaka University; Osaka, Japan;

Keywords: influenza vaccine, immunogenicity, reactogenicity, hematological malignancy, rituximab

Abbreviations: MFR, mean fold rise; GMT, geometric mean titer; sR, seroresponse proportion; sP, seroprotection proportion; HI, hemagglutination inhibition; sC, seroconversion proportion; EMA, European Medicines Agency; ORS, oculorespiratory syndrome; MDS, myelodysplatic syndrome; OR, odds ratios ; 95% CI, 95% confidence intervals

Patients with hematological malignancies have high risk for morbidity and mortality from influenza. This study was conducted to evaluate the immunogenicity and reactogenicity of an influenza A(H1N1)pdm09 vaccine among such subjects. Fifty subjects were vaccinated twice during the 2009–2010 season. The antibody response was expressed in terms of mean fold rise (MFR) of geometric mean titer, seroresponse proportion (sR), and seroprotection proportion (sP). The first vaccination induced only a small response, and additional antibody was acquired after the second dose (MFR 2.3 and 3.9, sR 32% and 54%, and sP 30% and 48% after the first and the second vaccination, respectively). Rituximab treatment showed an especially inhibitory effect (MFR 1.3, sR 9% and sP 0%). When analyzed using logistic regression models, only rituximab was found to have an independent effect; the adjusted odds ratio for sR was 0.09 ($P = 0.05$). Influenza vaccination of patients with hematological malignancies resulted in adepuate response, and the second vaccination induced additional antibody. It is therefore recommended to vaccinate this group twice.

Introduction

The United States Advisory Committee on Immunization Practices (US ACIP) recommends annual influenza vaccination for immunocompromised patients.[1-3] Patients with hematological malignancies have reduced immune response and therefore are at high risk for morbidity and mortality due to influenza.[4] It is said that their treatment with cytotoxic chemotherapy drugs induced a reduction of humoral response and led to increased susceptibility to infectious disease.[5-7] In fact, high mortality and morbidity of this population due to an influenza virus was reported.[8] On the other hand, it is unclear whether the underlying disease causes this lowered response. Thus more studies are required to evaluate the effectiveness of influenza vaccine in such patients and to protect this group from influenza. However there have been only a limited number of reports and they showed conflicting data.[9-16]

In March 2009, a novel influenza A(H1N1) virus was reported in North America.[17-19] This virus spread globally and brought about the 2009 influenza pandemic.[20-22] Because this virus was novel for human beings, we got an exceptional opportunity to study the immunogenicity of an influenza vaccine in a naive population. The objective of this study was to assess the immunogenicity and reactogenicity of a monovalent A(H1N1)pdm09 influenza vaccine in patients with hematological malignancies.

Results

Table 1 shows characteristics of the study subjects. The median age was 59 (range 21–83), and 48% of them were 60 or older. 40% of the subjects were males, 92% had pre-titer < 1:10, and 2 subjects had pre-titer ≥ 1:40 (1:40 and 1:80). Lymphoma was the most common underlying disease (42%). All lymphoma patients had non-Hodgkin's disease, and there was no patient with Hodgkin's disease. Steroid was the most frequently used chemotherapeutic agent (58%). Rituximab was being used on 11 (22%) patients only, but nearly half (48%) of the lymphoma patients were receiving rituximab.

*Correspondence to: Yuichiro Ide; Email: ideyuichiro3t3@gmail.com
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Table 1. Characteristics of patients with hematological malignancy

Characteristics	Patients (n = 50)	
Age Median (range)	59	(21–83)
Gender Male	20	(40)
Prevaccination titer < 1:10 1:10–1:20 ≥ 1:40	46 2 2	(92) (4) (4)
Underlying disease Lymphoma Acute Leukemia Myeloma MDS[a] Aplastic anemia Other [b]	21 14 8 3 2 2	(42) (28) (16) (6) (4) (4)
Chemotherapy [c] Steroid Immunosuppressive agent Anticancer agent [d] Rituximab	29 6 16 11	(58) (12) (32) (22)
Chemotherapy by Underlying disease Lymphoma Steroid Immunosuppressive agent Anticancer agent [d] Rituximab Acute leukemia Steroid Immunosuppressive agent Anticancer agent [d] Rituximab Myeloma Steroid	21 15 2 13 10 14 8 3 2 1 8 4	(100) (71) (10) (62) (48) (100) (57) (21) (14) (7) (100) (50)

Note: Number in parentheses is expressed as percentage if not otherwise specified; [a]MDS (myelodysplastic syndrome);[b]Other includes chronic myelogenous leukemia and myelofibrosis; [c]Each treatment is not mutually exclusive; [d]Anticancer agents does not include rituximab;

Immunogenicity of the vaccine is summarized in **Table 2**. In the entire sample, GMT did not reach the protective level (≥ 1:40) even after the second vaccination (S2 = 1:22). MFR reached 2.3 after the first and 3.9 after the second vaccination. sR became 32% and 54% and sP became 27% and 46% after the first and the second vaccination, respectively Females had 1/3 or less the GMT of males both after the first and the second vaccinations with clearly lower MFR, sR and sP. In the two categories with pre-titer ≥ 1:10 there was almost no increase in MFR, and both sR and sP were 0%. Patients with lymphoma, acute leukemia, or myeloma as the underlying disease showed significant increase in MFR after the second vaccination, but they differed in the extent of this increase (MFR of 2.0 for lymphoma, 4.6 for acute leukemia and 9.5 for myeloma). Similarly, the sR (S1/S0 = 10% and S2/S0 = 33%) and sP (S1 = 10% and S2 = 19%) were significantly low in lymphoma patients compared with patients with other underlying diseases. The values of various parameters of categories of patients who were under different types of chemotherapy were generally lower than those of the entire sample, irrespective of the drug used. The values were particularly low with rituximab, MFR, sR and sP being 1.3, 9% and 0% respectively after the second vaccination.

MFR after the second vaccination was 3.9 for the entire sample, which satisfied the EMA criterion (2.5). The two categories with pre-titer ≥ 1:10 both had sR 0% after the first and the second vaccinations, and sC also became 0%. Because of this, sC for the entire sample was 26% (13/50) after the first vaccination and 44% (22/50) after the second vaccination. Thus, as with MFR, the vaccine satisfied the EMA criterion (40%) for sC also for the entire sample.

Table 3 shows the results of logistic regression analysis of sR (MFR S2/S0 ≥ 4) after the second vaccination. In the univariate analysis, significantly reduced ORs were seen for lymphoma (P = 0.01), steroid (P = 0.02), anticancer agents (P = 0.02) and rituximab (P = 0.01), and there was marginal significance (P = 0.09) for gender. In the multivariate analysis of model 1, where age, gender and underlying disease were included, the OR for lymphoma showed marginal significance (P = 0.06), but there was no significant reduction in the OR for gender (P = 0.50). In model 2, where gender was not taken into account, the OR for lymphoma showed statistical significance (OR = 0.08, 95% CI = 0.01–0.95). In model 3, where age and chemotherapy were included, statistical significance was seen only for rituximab (OR = 0.08, 95% CI = 0.01–0.86). Finally, in model 4, which included age, lymphoma and rituximab, only rituximab showed significance, that too only marginal (OR = 0.09, 95% CI = 0.01–1.04). Lymphoma did not have significant effect (OR = 0.43, 95% CI = 0.08–2.18).

Table 4 shows the results for sP after the second vaccination (S2 ≥ 1:40). Significantly reduced ORs were seen in the univariate analysis for gender (P = 0.03), lymphoma (P = 0.01) and anticancer agents (P = 0.05), and there was marginal significance (P = 0.09) for myeloma. The antibody titer did not reach the seroprotective level in any of the patients under rituximab treatment. Therefore we could not include rituximab in the model. In the multivariate model 1, which included age, gender and underlying disease, the OR for lymphoma maintained a significance (P = 0.04) and the OR for gender was not significant (P = 0.32). In model 2, where gender was not taken into account, OR for lymphoma showed even greater decrease (OR = 0.07, 95% CI = 0.01–0.76). In model 3, where age and chemotherapy were included, no variable showed statistically significant OR. Finally in model 4, which included age, lymphoma and chemotherapy, only lymphoma showed a significant decrease in OR (OR = 0.10, 95% CI = 0.02–0.58).

We examined the associations among these explanatory variables by calculating Cramer's V. Gender and lymphoma had Cramer's V of 0.42 (P < 0.01). In fact, a higher proportion of females than males had lymphoma (3/20 males and 18/30 females). In the univariate analysis, females showed a lower OR because of this skew. Cramer's V was 0.53 (P < 0.001) between lymphoma and rituximab. When the frequency of rituximab treatment was compared between lymphoma and non-lymphoma patients, it was seen that mostly lymphoma patients had received the treatment (10/21 lymphoma patients and 1/29 non-lymphoma

Table 2. Immunogenicity of monovalent 2009 influenza A (H1N1) vaccine on hematological malignancy patients

		GMT					MFR						sR						sP					
													S1/S0 ≥ 4			S2/S0 ≥ 4			S1 ≥ 1:40			S2 ≥ 1:40		
Category	N	S0		S1		S2 n	S1/S0 (%) n			S2/S0 (%) n			(%)		n	(%)								
Entire sample	50	6		13		22	2.3			3.9			16	(32)		27	(54)		13	(27)		22	(46)	
Age 21–59 60–83	27 23	6 5		16 11		27 18	2.3 2.4	* *		4.3 3.2	* *		9 7	(33) (30)		15 12	(56) (52)		8 5	(32) (22)		12 10	(48) (44)	
Gender Male Female	20 30	7 5		26 8	†	43 14	†	4.0 1.6	* *	†	6.5 2.8	* *	†	10 6	(50) (20)	**	13 14	(65) (47)		8 5	(44) (17)	**	12 10	(67) (33)	**
Prevaccination titer < 1:10 1:10–1:20 ≥ 1:40	46 2 2	5 10 57	†	13 7 57		22 10 80	2.5 0.7 1.0	*	†	4.4 1.0 1.4	*		16 0 0	(35)		27 0 0	(59)		13 0 -	(28)		22 0 -	(48)	
Underlying disease Lymphoma Acute leukemia Myeloma MDS [a] Aplastic anemia Other [b]	21 14 8 3 2 2	5 6 5 5 20 5	†	7 13 20 40 20 320	†	10 30 48 40 40 160	†	1.4 2.0 4.0 8.0 1.0 64.0	* *	†	2.0 4.6 9.5 8.0 2.0 32.0	* * *	†	2 5 4 3 0 2	(10) (36) (50) (100) (100)	**	7 9 6 3 0 2	(33) (64) (75) (100) (100)	**	2 3 4 2 0 2	(10) (23) (50) (67) (100)	**	4 8 6 2 0 2	(19) (62) (75) (67) (100)	**
Chemotherapy [c] Steroid Immunosuppressive agent Anticancer agent [d] Rituximab	29 6 16 11	6 8 5 6		10 13 8 6	† †	18 25 11 8	† †	1.7 1.6 1.5 1.0	*	† † †	2.9 3.2 2.1 1.3	* *	† † †	6 1 2 0	(21) (17) (13)	‡ ‡	11 2 5 1	(38) (33) (31) (9)	‡ ‡ ‡	5 1 2 0	(19) (20) (13)		10 2 4 0	(37) (40) (25)	

Note: GMT (geometric mean titer); MFR (mean fold rise); sR (seroresponse proportion); sP (seroprotection proportion), those with S0 ≥ 1:40 were excluded; [a]MDS (myelodysplastic syndrome); [b]Other includes chronic myelogenous leukemia and myelofibrosis; [c]Each treatment is not mutually exclusive and comparisons were conducted with absent group; [d]Anticancer agents does not include rituximub;* $P < 0.05$ in Wilcoxon signed rank test for intra-category comparisons; † $P < 0.05$ in Wilcoxon rank sum test or Kruskal-Wallis test for inter-category comparisons; ** $P < 0.05$ in $\chi 2$ test; ‡ $P < 0.05$ in $\chi 2$ test (compared with patients who were not administrated corresponding chemotherapeutics).

Table 3. Association between selected characteristics and SeroResponse proportion (S0 to S2) (n = 46)

			Crude		Multivariate model 1[a]		Multivariate model 2[b]		Multivariate model 3[c]		Multivariate model 4[d]	
Category		sR (%)	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Age	60–83/36–59	52/60	0.73 (0.23–2.28)	0.59	1.58 (0.37–6.74)	0.53	1.52 (0.37–6.37)	0.56	0.71 (0.18–2.80)	0.63	0.96 (0.22–4.15)	0.95
Gender	Female/ Male	47/72	0.34 (0.10–1.18)	0.09	0.60 (0.14–2.63)	0.50						
Underlying disease Lymphoma Acute leukemia Myeloma	 +/- +/- +/–	 33/74 69/51 75/53	 0.18(0.05–0.61) 2.13 (0.55–8.21) 2.71 (0.49–15.1)	 0.01 0.27 0.25	 0.09 (0.01–1.12) 0.40 (0.03–4.83) 0.39 (0.02–6.96)	 0.06 0.47 0.52	 0.08 (0.01–0.95) 0.40 (0.03–4.76) 0.47 (0.03–7.71)	 0.05 0.47 0.59			 0.43 (0.08–2.18)	 0.31
Chemotherapy Steroid Immunosuppressive Agents Anticancer agents* Rituximab	 +/- +/- +/–	 41/76 40/58 30/69 10/68	 0.22 (0.06–0.76) 0.48 (0.07–3.17) 0.21 (0.06–0.75) 0.05 (0.01–0.45)	 0.02 0.45 0.02 0.01					 0.75 (0.11–4.93) 0.42 (0.04–4.49) 0.56 (0.08–3.84) 0.08 (0.01–0.86)	 0.76 0.47 0.55 0.04	 0.09 (0.01–1.04)	 0.05

Logistic regression model. CI: confidence interval; OR: odds ratio; [a]Model include age, sex, lymphoma, acute leukemia, and myeloma; [b]Model include age, lymphoma, acute leukemia and myeloma. [c]Model include age, steroid, immunosuppressive agents, anticancer agents and rituximab; [d]Model include age, lymphoma and rituximab; *Anticancer agents does not include rituximab.

patients). This strongly suggested that lymphoma maintained significant association in model 4 of **Table** 4 because this model did not include rituximab.

Table 5 shows the proportion of subjects who had adverse events. No mortality or serious adverse event was reported. Only 2 patients reported adverse events after the first vaccination. One patient (2%) had a systemic reaction while another (2%) had a localized reaction. No patient reported symptoms of ORS. On the other hand, this syndrome was reported after the second vaccination by 4 patients (8%). After the second vaccination, systemic reaction was reported by 12 (24%) patients and localized reaction by 10 (20%). However, all the adverse events were of grade 1.

Discussion

In the present study, we gave two vaccinations of an influenza A(H1N1)pdm vaccine to patients with hematological malignancies. The immunological indices of the subjects were considerably lower (MFR 3.9 times, sR 54% and sP 46%) than in healthy adults.[23-29] There have been quite a few reports about the low immunogenicity of influenza vaccines in patients with hematological malignancies.[30-38] The results obtained by use here agree with their findings. In healthy adults usually the induction of antibody reaches a plateau after one vaccination.[23-29] However in patients with hematological malignancies antibodies were found to be induced further even after the second vaccination. We recommend two vaccinations for such patients as an additional effect can be expected from the second vaccination. Very recent research on patients of lymphoid tumors has also revealed an additional effect of the second vaccination, and the authors of those studies have also recommended vaccinating such patients twice.[39,40]

Comparison of various immunological indices in patients stratified for various characteristics showed that the indices were low in females, lymphoma patients, and those treated with steroids, anticancer agents or rituximab (**Table 2**). The values were particularly low with rituximab, with only one patient showing a 4-fold increase in antibody titer even after two vaccinations, and none reaching titer ≥ 1:40. Recent studies have also shown the low immunogenicity of influenza vaccines in lymphoma patients and patients on rituximab treatment.[30-43] The results obtained by us here are in agreement with those findings. Subjects having pre-titer ≥ 1:10 did not show any increase in antibody response after the second vaccination. This may have happened by chance, because such subjects were very few. Larger studies are needed to confirm this.

In the present study we performed multivariate logistic analysis only with subjects having pre-titer < 1:10 in order to eliminate the effect of pre-titer level on antibody induction. Pre-titer is an important factor in immune response and this calls for special

Table 4. Association between selected characteristics and SeroProtection proportion (after S2) (n = 46)

			Crude		Multivariate model 1[a]		Multivariate model 2[b]		Multivariate model 3[c]		Multivariate model 4[d]	
Category		sR (%)	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Age	60–83/36–59	44/48	0.83 (0.27–2.60)	0.75	2.14 (0.42–10.8)	0.36	1.98 (0.41–9.60)	0.40	0.93 (0.28–3.11)	0.91	2.02 (0.44–9.26)	0.37
Gender	Female/ Male	33/67	0.25 (0.07–0.86)	0.03	0.48 (0.11–2.06)	0.32						
Underlying disease Lymphoma Acute leukemia Myeloma	 +/- +/- +/–	 19/68 62/40 75/40	0.12 (0.03–0.46) 2.40 (0.65–8.86) 4.50 (0.81–25.2)	 0.01 0.19 0.09	0.09 (0.01–0.94) 0.66 (0.08–5.46) 0.78 (0.06–10.4)	 0.04 0.70 0.85	0.07 (0.01–0.76) 0.66 (0.08–5.27) 1.01 (0.08–12.2)	 0.03 0.69 1.00			0.10 (0.02–0.58)	0.01
Chemotherapy Steroid Immunosuppressive Agents Anticancer agents* Rituximab	 +/- +/- +/–	 37/57 40/47 25/56 0/58	0.44 (0.14–1.41) 0.77 (0.12–5.06) 0.26 (0.07–0.98) NA	 0.17 0.78 0.05					1.11 (0.20–6.08) 0.57 (0.06–5.51) 0.23 (0.04–1.43)	 0.91 0.63 0.12	0.69 (0.10–4.71) 0.88 (0.08–9.99) 0.96 (0.10–9.12)	 0.70 0.92 0.97

Logistic regression model. CI: confidence interval; OR: odds ratio; [a]Model include age, sex, lymphoma, acute leukemia, and myeloma; [b]Model include age, lymphoma, acute leukemia and myeloma; [c]Model include age, steroid, immunosuppressive agents and anticancer agents; [d]Model include age, lymphoma and anticancer agents;*Anticancer agents does not include rituximab.

care in the analysis of the data.[44,45] Here we used a vaccine against the A(H1N1)pdm09 influenza strain, which is a novel strain to which most people had not been exposed. Therefore, very few subjects (n = 4) had the antibody (titer ≥ 1:10) before the vaccination. Exclusion of these subjects from the analysis did not have a major effect.

Gender-stratified ORs, which showed significance in univariate analysis, lost their significance in the multivariate analysis of sR and sP when lymphoma was simultaneously included in the analysis, the ORs nearly reaching the value of 1 (**Tables 3 and 4**). On the other hand, the ORs for sR and sP adjusted for lymphoma which simultaneously took the gender also into account showed strong reduction. This suggested that the significance of the gender-stratified ORs seen here was because of association with lymphoma. Among the chemotherapies, only the adjusted OR for rituximab showed significance (**Table 3**), and there was an association between rituximab treatment and lymphoma. As with gender there was the possibility of the effect being due to the association with lymphoma. We therefore simultaneously adjusted for rituximab and lymphoma in the final model. As a result, lymphoma's OR for sR became close to 1 whereas rituximab's OR remained about the same. This suggests that not lymphoma but rituximab was blocking the immune response. The inhibitory effect of rituximab on antibody induction has been reported by many, but we believe that ours is the first report that demonstrates this effect by eliminating the effect of the underlying disease (lymphoma), through multivariate logistic regression analysis.[19,39-42] There is a possibility that the observed effect was related to the fact that the multivariate logistic regression analysis did not include Hodgkin's lymphoma. The Japanese population has fewer patients of Hodgkin's lymphoma than other populations, and our patients also did not have this disease. Future studies with other racial population are required to resolve this.

Rituximab is a monoclonal antibody that specifically recognizes the CD20 antigen and induces phagocytosis of B cells.[46,47] The CD20 antigen is expressed on malignant B cells and also on mature B cells.[48,49] Therefore, administration of rituximab causes destruction of malignant B cells as well as mature B cells, and persons under rituximab treatment show depletion of B cells. This type of B cell depletion can persist for long periods of time. It has been reported that even patients who had been under complete remission for long (≥ 6 mo) had low ability to induce antibodies against influenza vaccines.[41,50] After receiving rituximab treatment such patients do not attain the optimum antibody titer through influenza vaccination for a long time. Therefore, we need to inform people who come into close contact with such patients to get vaccinated for influenza and to adopt other measures to prevent the spread of the infection to them.

There was no report of mortality or any serious adverse events after the vaccination in the present study. All the adverse events

Table 5. Reactgenicity of patients with hematological malignancy

	after first vaccination		after second vaccination	
Oculorespiratory syndrome [a]	0		4	(8)
Any	0		2	(4)
Red eyes	0		1	(2)
Facial edema	0		3	(6)
Respiratory symptoms				
Systematic reactions [b]	1		12	(24)
Any	1		3	(6)
Feaver (> 37 °C)	0	(2)	8	(16)
Malaise	0	(2)	5	(10)
Myalgia	0		8	(16)
Headache	0		1	(2)
Rash				
Local reactions [b]	1		10	(20)
Any	0		7	(14)
Redness	0	(2)	3	(6)
Swelling	1	(2)	3	(6)
Induration	1	(2)	5	(10)
Itching	0		1	(2)
Pain				

Note: Number in parentheses is expressed as percentage; [a]symptoms within 24 h after vaccination; [b]symptoms within 48 h after vaccination.

reported were common ones and of grade 1, suggesting that the vaccine was tolerated well.

In this study, we have shown adequate immunogenicity and good tolerance of the A(H1N1)pdm09 vaccine and demonstrated the effect of two dose vaccination of immunocompromised patients by using epidemiological methods. Unfortunately, our multivariate model suggested that rituximab treatment had an inhibitory effect on the immune response.

This study was conducted at the time of the influenza pandemic season. The data of immunogenicity and reactogenicity of the vaccine was required urgently. So study subjects were limited. Thus we could not include pre-vaccination immune function and pre-existing medical conditions other than malignancies in the analysis. Our present limited study yielded the aforesaid results. Future multicenter studies may provide more compelling evidence.

To sum up, various antibody indices measured after a second vaccination with an influenza A(H1N1)pdm09 vaccine were adequate in patients of hematological malignancies, and all the EMA criteria were satisfied. Furthermore because the second vaccination showed an additional effect, we recommend that such patients be vaccinated twice. Multivariate logistic analysis showed that rituximab interfered with immunogenicity of the influenza vaccine. Thus it is necessary to pay attention to the fact that vaccination of patients under rituximab treatment could possibly result in failure to achieve the required antibody levels.

Materials and Methods

Subjects

We recruited 50 patients with hematological malignancies from St. Mary's hospital in Fukuoka, Japan during October 2009. In Japan, a pdm09 strain was first reported in May, and the epidemic reached its peak in November. A similar trend was observed at the study location. Exclusion criteria were post-partial remission malignancy, fever of over 38 °C, history of past vaccination allergy, known allergy to egg products, and bleeding tendency due to DIC. This study was approved by the ethics review committees of the Osaka City University, St. Mary's College and St. Mary's hospital. Written informed consents were obtained from the patients or their guardians.

Vaccination and HI assay

The monovalent unadjuvanted inactive A(H1N1) pdm09 split-virus vaccine (Lot.HP01A: BIKEN, Osaka, Japan) contains 30μg/mL of hemagglutinin [A/California/7/2009 (H1N1)]. 0.5 ml of the vaccine was administered subcutaneously twice, 4 wk apart. Blood samples were drawn at baseline (S0), 4 wk after each of the first vaccination (S1) and the second vaccination (S2). All serum samples were stored at -80 °C until used. Hemagglutination inhibition (HI) assay was conducted as described previously.[51]

Information collection

Information about underlying disease (disease name) and chemotherapy (whether administered and duration) was obtained from medical charts. Frequency and severity of adverse events were examined using self-administrated questionnaires on oculorespiratory syndrome (ORS) (within 24h) and systemic reactions and local reactions (within 48 h).[3] All adverse events were graded as follows: grade 1 (present but not interfere with dairy activities), grade 2 (moderate) and grade 3 (prevents daily activities).[19] We also collected information about ORS, because it has been reported occasionally within 24 h after seasonal trivalent influenza vaccination.[52,53] Serious adverse events were defined as reports of death, life-threatening illness, hospitalization or prolongation of hospitalization, or permanent disability, according to the Vaccine Adverse Events Reporting System (VAERS).[54]

Statistical analysis

The antibody response was assessed by calculating the following indices: mean fold rise (MFR) of geometric mean titer (GMT), seroresponse proportion (sR, the proportion of subjects showing ≥ 4-fold rise) and seroprotection proportion (sP, the proportion with postvaccination titer ≥ 1:40).[55] We also calculated seroconversion proportion (sC, proportion with baseline titer < 1:10 and postvaccination titer ≥ 1:40 or baseline titer ≥ 1:10 and ≥ 4-fold rise), and compared our results with the European Medicines Agency (EMA) criteria (sC > 40%, MFR > 2.5 and sP > 70%).[56]

For data processing, HI titer < 1:10 was regarded as 1:5. Reciprocal titers were used for analyses after logarithmic transformation. The results were presented on the original scale by calculating the antilogarithms. Wilcoxon signed-rank test was applied for intracategory comparisons of MFR, and either Wilcoxon rank-sum test or Kruskal-Wallis test was used for intercategory comparisons of GMT. Chi-square test or Mantel-extension test was performed, as appropriate, for comparisons of sR and sP.

Antibody response was assessed for populations stratified for age, gender, underlying disease and type of chemotherapy. Cyclosporine and tacrolimus were grouped with immunosuppressive agents. The categories of chemotherapy were not exclusive.

We conducted multivariate logistic regression analysis to examine the effect of each factor on objective variables (sR and sP after second dose). This multivariate analysis was limited to patients with prevaccination antibody titer (pre-titer) < 1:10 (n = 46). Myelodysplatic syndrome (MDS), aplastic anemia and other disease were not included in the models as there were only a few such patients (n = 3, 2 and 2, respectively). Multivariate models were analyzed in two separate steps because of the limited number of subjects. Models of the first step analysis included underlying disease, and those of the second step analysis included chemotherapeutic agents, as explanatory variables, along with age and gender. The final model included factors selected in each previous analysis step to enable identification of the factors that were more prominently associated with the lowered immune response, from among the underlying medical conditions and treatments adopted for those conditions. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. We also calculated Cramer's V to detect relationships among the variables. A two-sided *P* value of less than 0.05 was considered statistically significant. A *P* value less than 0.10 and larger than or equal to 0.05 was regarded as marginally significant.

All statistical analyses were performed using SAS ver. 9.3 (SAS institute, NC, USA).

Disclosures

No conflict of interest statement declared.

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References

1. Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, Mootrey G, Bresee JS, Cox NJ; Centers for Disease Control and Prevention. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. MMWR Recomm Rep 2009; 58(RR-8):1-52; PMID:19644442
2. Fiore AE, Uyeki TM, Broder K, Finelli L, Euler GL, Singleton JA, Iskander JK, Wortley PM, Shay DK, Bresee JS, et al.; Centers for Disease Control and Prevention (CDC). Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. MMWR Recomm Rep 2010; 59(RR-8):1-62; PMID:20689501
3. Centers for Disease Control and Prevention (CDC). Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2011. MMWR Morb Mortal Wkly Rep 2011; 60:1128-32; PMID:21866086
4. Hodges GR, Davis JW, Lewis HD Jr., Whittier FC Jr., Siegel CD, Chin TD, Clark GM, Noble GR. Response to influenza A vaccine among high-risk patients. South Med J 1979; 72:29-32; PMID:366766; http://dx.doi.org/10.1097/00007611-197901000-00010
5. Chemaly RF, Ghosh S, Bodey GP, Rohatgi N, Safdar A, Keating MJ, Champlin RE, Aguilera EA, Tarrand JJ, Raad II. Respiratory viral infections in adults with hematologic malignancies and human stem cell transplantation recipients: a retrospective study at a major cancer center. Medicine (Baltimore) 2006; 85:278-87; PMID:16974212; http://dx.doi.org/10.1097/01.md.0000232560.22098.4e
6. Vidal L, Gafter-Gvili A, Leibovici L, Dreyling M, Ghielmini M, Hsu Schmitz SF, Cohen A, Shpilberg O. Rituximab maintenance for the treatment of patients with follicular lymphoma: systematic review and meta-analysis of randomized trials. J Natl Cancer Inst 2009; 101:248-55; PMID:19211444; http://dx.doi.org/10.1093/jnci/djn478
7. Salles G, Seymour JF, Offner F, López-Guillermo A, Belada D, Xerri L, Feugier P, Bouabdallah R, Catalano JV, Brice P, et al. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet 2011; 377:42-51; PMID:21176949; http://dx.doi.org/10.1016/S0140-6736(10)62175-7
8. Liu C, Schwartz BS, Vallabhaneni S, Nixon M, Chin-Hong PV, Miller SA, Chiu C, Damon L, Drew WL. Pandemic (H1N1) 2009 infection in patients with hematologic malignancy. Emerg Infect Dis 2010; 16:1910-7; PMID:21122221; http://dx.doi.org/10.3201/eid1612.100772
9. Mazza JJ, Yale SH, Arrowood JR, Reynolds CE, Glurich I, Chyou PH, Linneman JG, Reed KD. Efficacy of the influenza vaccine in patients with malignant lymphoma. Clin Med Res 2005; 3:214-20; PMID:16303886; http://dx.doi.org/10.3121/cmr.3.4.214
10. Monkman K, Mahony J, Lazo-Langner A, Chin-Yee BH, Minuk LA. The pandemic H1N1 influenza vaccine results in low rates of seroconversion for patients with hematological malignancies. Leuk Lymphoma 2011; 52:1736-41; PMID:21663502; http://dx.doi.org/10.3109/10428194.2011.584003
11. Bate J, Yung CF, Hoschler K, Sheasby L, Morden J, Taj M, Heath PT, Miller E. Immunogenicity of pandemic (H1N1) 2009 vaccine in children with cancer in the United Kingdom. Clin Infect Dis 2010; 51:e95-104; PMID:21067352; http://dx.doi.org/10.1086/657403
12. Brydak LB, Machała M, Centkowski P, Warzocha K, Biliński P. Humoral response to hemagglutinin components of influenza vaccine in patients with non-Hodgkin malignant lymphoma. Vaccine 2006; 24:6620-3; PMID:16870313; http://dx.doi.org/10.1016/j.vaccine.2006.05.100
13. Porter CC, Edwards KM, Zhu Y, Frangoul H. Immune responses to influenza immunization in children receiving maintenance chemotherapy for acute lymphoblastic leukemia. Pediatr Blood Cancer 2004; 42:36-40; PMID:14752792; http://dx.doi.org/10.1002/pbc.10459
14. Ljungman P, Avetisyan G. Influenza vaccination in hematopoietic SCT recipients. Bone Marrow Transplant 2008; 42:637-41; PMID:18724396; http://dx.doi.org/10.1038/bmt.2008.264
15. Ljungman P, Nahi H, Linde A. Vaccination of patients with haematological malignancies with one or two doses of influenza vaccine: a randomised study. Br J Haematol 2005; 130:96-8; PMID:15982350; http://dx.doi.org/10.1111/j.1365-2141.2005.05582.x
16. Pollyea DA, Brown JM, Horning SJ. Utility of influenza vaccination for oncology patients. J Clin Oncol 2010; 28:2481-90; PMID:20385981; http://dx.doi.org/10.1200/JCO.2009.26.6908
17. Centers for Disease Control and Prevention (CDC). Novel influenza A (H1N1) virus infections in three pregnant women - United States, April-May 2009. MMWR Morb Mortal Wkly Rep 2009; 58:497-500; PMID:19444154
18. Smith GJ, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, Ma SK, Cheung CL, Raghwani J, Bhatt S, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature 2009; 459:1122-5; PMID:19516283; http://dx.doi.org/10.1038/nature08182
19. Mackay HJ, McGee J, Villa D, Gubbay JB, Tinker LM, Shi L, Kuruvilla J, Wang L, MacAlpine K, Oza AM. Evaluation of pandemic H1N1 (2009) influenza vaccine in adults with solid tumor and hematological malignancies on active systemic treatment. J Clin Virol 2011; 50:212-6; PMID:21168361; http://dx.doi.org/10.1016/j.jcv.2010.11.013
20. Centers for Disease Control and Prevention (CDC). Outbreak of swine-origin influenza A (H1N1) virus infection - Mexico, March-April 2009. MMWR Morb Mortal Wkly Rep 2009; 58:467-70; PMID:19444150
21. Gordon SM. Update on 2009 pandemic influenza A (H1N1) virus. Cleve Clin J Med 2009; 76:577-82; PMID:19797457; http://dx.doi.org/10.3949/ccjm.76a.05009
22. Kao TM, Wang CH, Chen YC, Ko WJ, Chang SC. The first case of severe novel H1N1 influenza successfully rescued by extracorporeal membrane oxygenation in Taiwan. J Formos Med Assoc 2009; 108:894-8; PMID:19933034; http://dx.doi.org/10.1016/S0929-6646(09)60422-8
23. Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, Dawson G, Hu W, Leggio C, Washington D, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. N Engl J Med 2009; 361:2405-13; PMID:19745216; http://dx.doi.org/10.1056/NEJMoa0907413
24. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, Stephenson I. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. N Engl J Med 2009; 361:2424-35; PMID:19745215; http://dx.doi.org/10.1056/NEJMoa0907650

25. Liang XF, Wang HQ, Wang JZ, Fang HH, Wu J, Zhu FC, Li RC, Xia SL, Zhao YL, Li FJ, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2010; 375:56-66; PMID:20018364; http://dx.doi.org/10.1016/S0140-6736(09)62003-1
26. Plennevaux E, Sheldon E, Blatter M, Reeves-Hoché MK, Denis M. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. Lancet 2010; 375:41-8; PMID:20018365; http://dx.doi.org/10.1016/S0140-6736(09)62026-2
27. Roman F, Vaman T, Gerlach B, Markendorf A, Gillard P, Devaster JM. Immunogenicity and safety in adults of one dose of influenza A H1N1v 2009 vaccine formulated with and without AS03A-adjuvant: preliminary report of an observer-blind, randomised trial. Vaccine 2010; 28:1740-5; PMID:20034605; http://dx.doi.org/10.1016/j.vaccine.2009.12.014
28. Vajo Z, Tamas F, Sinka L, Jankovics I. Safety and immunogenicity of a 2009 pandemic influenza A H1N1 vaccine when administered alone or simultaneously with the seasonal influenza vaccine for the 2009-10 influenza season: a multicentre, randomised controlled trial. Lancet 2010; 375:49-55; PMID:20018367; http://dx.doi.org/10.1016/S0140-6736(09)62039-0
29. Zhu FC, Wang H, Fang HH, Yang JG, Lin XJ, Liang XF, Zhang XF, Pan HX, Meng FY, Hu YM, et al. A novel influenza A (H1N1) vaccine in various age groups. N Engl J Med 2009; 361:2414-23; PMID:19846844; http://dx.doi.org/10.1056/NEJMoa0908535
30. Hodges GR, Davis JW, Lewis HD Jr., Whittier FC Jr., Siegel CD, Chin TD, Clark GM, Noble GR. Response to influenza A vaccine among high-risk patients. South Med J 1979; 72:29-32; PMID:366766; http://dx.doi.org/10.1097/00007611-197901000-00010
31. Mazza JJ, Yale SH, Arrowood JR, Reynolds CE, Glurich I, Chyou PH, Linneman JG, Reed KD. Efficacy of the influenza vaccine in patients with malignant lymphoma. Clin Med Res 2005; 3:214-20; PMID:16303886; http://dx.doi.org/10.3121/cmr.3.4.214
32. Monkman K, Mahony J, Lazo-Langner A, Chin-Yee BH, Minuk LA. The pandemic H1N1 influenza vaccine results in low rates of seroconversion for patients with hematological malignancies. Leuk Lymphoma 2011; 52:1736-41; PMID:21663502; http://dx.doi.org/10.3109/10428194.2011.584003
33. Bate J, Yung CF, Hoschler K, Sheasby L, Morden J, Taj M, Heath PT, Miller E. Immunogenicity of pandemic (H1N1) 2009 vaccine in children with cancer in the United Kingdom. Clin Infect Dis 2010; 51:e95-104; PMID:21067352; http://dx.doi.org/10.1086/657403
34. Brydak LB, Machała M, Centkowski P, Warzocha K, Biliński P. Humoral response to hemagglutinin components of influenza vaccine in patients with non-Hodgkin malignant lymphoma. Vaccine 2006; 24:6620-3; PMID:16870313; http://dx.doi.org/10.1016/j.vaccine.2006.05.100
35. Porter CC, Edwards KM, Zhu Y, Frangoul H. Immune responses to influenza immunization in children receiving maintenance chemotherapy for acute lymphoblastic leukemia. Pediatr Blood Cancer 2004; 42:36-40; PMID:14752792; http://dx.doi.org/10.1002/pbc.10459
36. Ljungman P, Avetisyan G. Influenza vaccination in hematopoietic SCT recipients. Bone Marrow Transplant 2008; 42:637-41; PMID:18724396; http://dx.doi.org/10.1038/bmt.2008.264
37. Ljungman P, Nahi H, Linde A. Vaccination of patients with haematological malignancies with one or two doses of influenza vaccine: a randomised study. Br J Haematol 2005; 130:96-8; PMID:15982350; http://dx.doi.org/10.1111/j.1365-2141.2005.05582.x
38. Pollyea DA, Brown JM, Horning SJ. Utility of influenza vaccination for oncology patients. J Clin Oncol 2010; 28:2481-90; PMID:20385981; http://dx.doi.org/10.1200/JCO.2009.26.6908
39. Bedognetti D, Ansaldi F, Zanardi E, Durando P, Sertoli MR, Massucco C, Balleari E, Racchi O, Zoppoli G, Orsi A, et al. Seasonal and pandemic (A/H1N1 2009) MF-59-adjuvanted influenza vaccines in complete remission non-Hodgkin lymphoma patients previously treated with rituximab containing regimens. Blood 2012; 120:1954-7; PMID:22936740; http://dx.doi.org/10.1182/blood-2012-06-438689
40. Villa D, Gubbay J, Sutherland DR, Laister R, McGeer A, Cooper C, Fortuno ES 3rd, Xu W, Shi L, Kukreti V, et al. Evaluation of 2009 pandemic H1N1 influenza vaccination in adults with lymphoid malignancies receiving chemotherapy or following autologous stem cell transplant. Leuk Lymphoma 2013; 54:1387-95; PMID:23240909; http://dx.doi.org/10.3109/10428194.2012.742524
41. Yri OE, Torfoss D, Hungnes O, Tierens A, Waalen K, Nordøy T, Dudman S, Kilander A, Wader KF, Ostenstad B, et al. Rituximab blocks protective serologic response to influenza A (H1N1) 2009 vaccination in lymphoma patients during or within 6 months after treatment. Blood 2011; 118:6769-71; PMID:22058114; http://dx.doi.org/10.1182/blood-2011-08-372649
42. Hottinger AF, George AC, Bel M, Favet L, Combescure C, Meier S, Grillet S, Posfay-Barbe K, Kaiser L, Siegrist CA, et al.; H1N1 Study Group. A prospective study of the factors shaping antibody responses to the AS03-adjuvanted influenza A/H1N1 vaccine in cancer outpatients. Oncologist 2012; 17:436-45; PMID:22357731; http://dx.doi.org/10.1634/theoncologist.2011-0342
43. Takata T, Suzumiya J, Ishikawa T, Takamatsu Y, Ikematsu H, Tamura K. Attenuated antibody reaction for the primary antigen but not for the recall antigen of influenza vaccination in patients with non-Hodgkin B-cell lymphoma after the administration of rituximab-CHOP. J Clin Exp Hematop 2009; 49:9-13; PMID:19474512; http://dx.doi.org/10.3960/jslrt.49.9
44. Hirota Y, Kaji M, Ide S, Goto S, Oka T. The hemagglutination inhibition antibody responses to an inactivated influenza vaccine among healthy adults: with special reference to the prevaccination antibody and its interaction with age. Vaccine 1996; 14:1597-602; PMID:9032887; http://dx.doi.org/10.1016/S0264-410X(96)00153-3
45. Kobayashi M, Ohfuji S, Fukushima W, Maeda A, Maeda K, Fujioka M, Hirota Y. Immunogenicity and reactogenicity of a monovalent inactivated 2009 influenza A vaccine in adolescents: with special reference to pre-existing antibody. J Pediatr 2012; 160:632-7, e1; PMID:22094234; http://dx.doi.org/10.1016/j.jpeds.2011.09.055
46. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, Newman RA, Hanna N, Anderson DR. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994; 83:435-45; PMID:7506951
47. Maloney DG, Liles TM, Czerwinski DK, Waldichuk C, Rosenberg J, Grillo-Lopez A, Levy R. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood 1994; 84:2457-66; PMID:7522629
48. Valentine MA, Meier KE, Rossie S, Clark EA. Phosphorylation of the CD20 phosphoprotein in resting B lymphocytes. Regulation by protein kinase C. J Biol Chem 1989; 264:11282-7; PMID:2472394
49. Press OW, Appelbaum F, Ledbetter JA, Martin PJ, Zarling J, Kidd P, Thomas ED. Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. Blood 1987; 69:584-91; PMID:3492224
50. Bedognetti D, Zoppoli G, Massucco C, Zanardi E, Zupo S, Bruzzone A, Sertoli MR, Balleari E, Racchi O, Messina M, et al. Impaired response to influenza vaccine associated with persistent memory B cell depletion in non-Hodgkin's lymphoma patients treated with rituximab-containing regimens. J Immunol 2011; 186:6044-55; PMID:21498665; http://dx.doi.org/10.4049/jimmunol.1004095
51. Organization WH. World Health Organization manual on animal influenza diagnosis and surveillance. Global Influenza Programme Geneva: WHO 2002.
52. Spila-Alegiani S, Salmaso S, Rota MC, Tozzi AE, Raschetti R. Reactogenicity in the elderly of nine commercial influenza vaccines: results from the Italian SVEVA study. Study for the evaluation of adverse events of influenza vaccination. Vaccine 1999; 17:1898-904; PMID:10217587; http://dx.doi.org/10.1016/S0264-410X(98)00467-8
53. Oculo-respiratory syndrome following influenza vaccination: review of post-marketing surveillance through four influenza seasons in Canada. Can Commun Dis Rep 2005; 31:217-25; PMID:16669126
54. Food and Drug Administration. 21 CFR Part 600.80. Postmarketing reporting of adverse experiences. Fed Regist 1997; 62:52252-3
55. Ohfuji S, Fukushima W, Deguchi M, Kawabata K, Yoshida H, Hatayama H, Maeda A, Hirota Y. Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine among pregnant women: lowered antibody response by prior seasonal vaccination. J Infect Dis 2011; 203:1301-8; PMID:21459817; http://dx.doi.org/10.1093/infdis/jir026
56. Guideline on influenza vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside the core dossier context. 2007.

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Original Article

Effectiveness of influenza vaccine in children in day-care centers of Sapporo

Mitsuru Mori,[1] Junko Hasegawa,[1] Satoko Showa,[1] Aiko Matsushima,[1] Hirofumi Ohnishi,[1] Yuko Yoto[2] and Hiroyuki Tsutsumi[2]
Departments of [1]Public Health, and [2]Pediatrics, Sapporo Medical University School of Medicine, Sapporo, Japan

Abstract ***Background***: We conducted a retrospective cohort study for evaluating the effectiveness of the trivalent inactivated influenza vaccine (TIV) among children aged 0–6 years in the 2011–2012 season in Sapporo City, Japan, because of scarce evidence.

Methods: From 10 day-care centers in Sapporo City, Japan, 629 parents participated in the study. Each parent of the subjects described whether a subject received TIV once or twice in the 2011–2012 season, as well as the exact dates of receiving TIV from records in a maternal and child health handbook marked by a pediatrician. The incidence of influenza was defined as being affected with influenza as diagnosed by a pediatrician. Cox's proportional model was used for calculating a hazard ratio (HR) and its 95% confidence interval (95%CI) of TIV on an influenza incidence.

Results: After adjusting potential confounding variables, such as the day-care center, presence of comorbidity, size of household, number of siblings, and number of smokers in the home in addition to the age and sex of the child, HR was significantly reduced in the subjects aged 1 year (HR = 0.22, 95%CI 0.09–0.54) as well as in the total subjects (HR = 0.72, 95%CI 0.52–0.99). Consequently, the effectiveness of TIV was calculated as 78% for the subjects aged 1 year and 28% for the total subjects.

Conclusion: Our study suggests that TIV is effective, especially in subjects aged 1 year. Further studies are necessary in different seasons, places, and populations to clarify the effectiveness of the influenza vaccine in children.

Key words children, effectiveness, influenza vaccine, retrospective cohort studies.

The influenza virus causes annual epidemics in the winter season in Japan, and it has been stated that vaccination against influenza in children should be promoted to prevent influenza-associated encephalitis-encephalopathy.[1] Increased awareness of the importance of influenza infection in children has led to an increase in the use of the influenza vaccine in Japan.[2] Trivalent inactivated vaccine (TIV) is now used every year for children in Japan.

According to the recent definition of vaccine efficacy and effectiveness,[3,4] efficacy is best measured by randomized controlled trials (RCT), and effectiveness is usually measured by observational studies. Efficacy or effectiveness of the live attenuated vaccine,[5–9] as well as the inactivated vaccine,[10–14] has been reported around the world. An RCT of the influenza vaccine in children aged 6–59 months showed superior efficacy of the live attenuated vaccine, as compared with the inactivated vaccine.[15] However, this trial also showed a higher rate of hospitalization for any cause among children aged 6–11 months in the live-attenuated-vaccine group than in the inactivated-vaccine group.[15] Other RCT of the influenza vaccine showed similar efficacy of the inactivated vaccine to the live attenuated vaccine in children aged 1–16 years[16] and in school children aged 9–12 years.[17]

Several RCT[10,11] or cohort studies[12–14] have shown significant efficacy or effectiveness of TIV to reduce the incidence of influenza in children. However, efficacy or effectiveness of TIV in children less than 3 years old is scarce in evidence and even controversial.[12,14] Accordingly, a retrospective cohort study was conducted for evaluating the effectiveness of TIV among children aged 0–6 years in the 2011–2012 season in Sapporo City, Japan.

Methods

Every large day-care center was identified from 10 districts in Sapporo. Then, 1570 parents of children attending these 10 day-care centers were invited to participate in the survey, and eventually, 629 parents (40.1%) gave written, informed consent to participate in this survey. Age distribution of the study subjects at the end of April 2012, was as follows: 43 were 0 years old, 122 were 1 year old, 127 were 2 years old, 119 were 3 years old, 106 were 4 years old, and 112 were 5 or 6 years old. A self-administered and structured questionnaire was distributed to their parents at the end of April 2012, and they returned a filled-out questionnaire in May by mail. Each parent described whether a subject received TIV once or twice in the 2011–2012 season, and if so, we noted the exact dates of receiving TIV according to records in a maternal and child health handbook marked by a

Correspondence: Mitsuru Mori, MD PhD, Department of Public Health, Sapporo Medical University School of Medicine, South 1, West 17, Chuo-ku, Sapporo 060-8556, Japan. Email: mitsurum@sapmed.ac.jp

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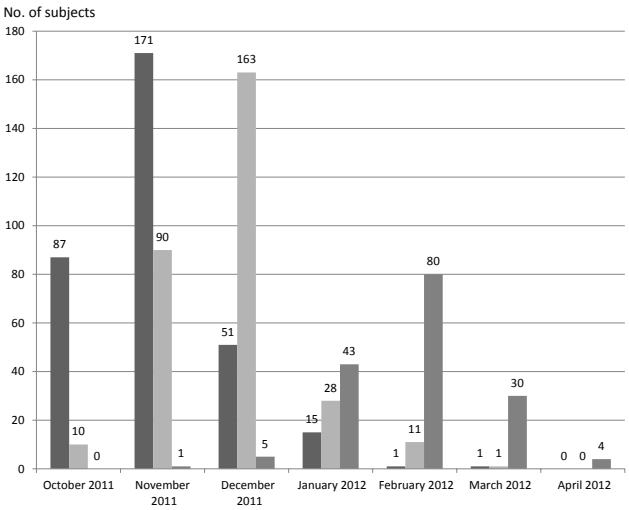

Fig 1. Distributions of the subjects in the first and second vaccinations of the trivalent inactivated vaccine and the incidence of influenza according to each month in the 2011–2012 season. ■, The first vaccination; ■, the second vaccination; ■, incidence of influenza.

pediatrician. TIV consisted of A/California/7/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Prisbane in the 2011–2012 season.[18] In addition, the questionnaire included inquiries about age, sex, size of household, number of siblings, number of smokers in the home, and so on.

The incidence of influenza was defined as being affected with influenza as diagnosed by a pediatrician. The exact date of the visit to a pediatrician and the name of the medical institute where the pediatrician worked were also obtained with the questionnaire. Cox's proportional model was used for calculating a hazard ratio (HR) and its 95% confidence interval (95%CI) of TIV on the influenza incidence. The start and end of observations were set at 1 October 2011, and 30 April 2012, respectively. SAS version 9.2 (SAS Institute, Cary, NC, USA) was utilized for every analysis. The significance level was set at 5%. This study was approved by the Ethical Committee of Sapporo Medical University (Approval date, 28 March 2012; Approval number, 23-2-76).

Results

From October 2011 to March 2012, 324 subjects among 629 participants (51.5%) received TIV at least once, and they were classified into the Vaccine group. In the Vaccine group, 302 subjects (93.2%) were fully vaccinated with two doses. As shown in Figure 1, the distribution of the subjects for the first vaccination according to months in the 2011–2012 season was as follows: 87 in October, 171 in November, 51 in December, 15 in January, one in February, and one in March. Furthermore, the distribution of the subjects on the second vaccination according to each month in the 2011–2012 season was as follows: 10 in October, 90 in November, 163 in December, 28 in January, 11 in February, and one in March.

Table 1 shows baseline characteristics of the subjects according to the status of receiving TIV, namely, the Vaccine and No vaccine groups. A subject who was vaccinated after being affected with influenza was classified into the No vaccine group. Various kinds of comorbidity were reported from 123 study children, including otitis media in 37 children and atopy or allergy in 19 children as the two most common comorbidities. The average age, proportion of boys, and presence of comorbidity were not different between the Vaccine and No vaccine groups. However, the distribution of day-care centers, size of the household, number of siblings, and number of smokers in the home were all significantly different between the two groups.

In the 2010–2011 season, 163 subjects (25.9%) were diagnosed as being affected with influenza by a pediatrician. As shown in Figure 1, the distribution of the subjects at the diagnosis of influenza according to months in the 2011–2012 season was as follows: one in November, five in December, 43 in January, 80 in February, 30 in March, and four in April.

Table 2 shows the sex-adjusted HR of TIV on the influenza incidence stratified by age. HR was significantly reduced in the subjects aged 1 year (relative risk = 0.24, 95%CI 0.10–0.56). Furthermore, sex- and age-adjusted HR were significantly

Table 1 Baseline characteristics of the subjects according to status of trivalent inactive vaccine in 2011–2012 season

Items	Vaccine group (n = 324)		No vaccine group (n = 305)		P-value
Age in years (mean, SD)	2.72	1.43	2.78	1.74	0.629
Boys (n, %)	155	47.8	149	48.9	0.799
Day-care center 1 (n, %)	44	13.6	19	6.2	<0.001
Day-care center 2 (n, %)	34	10.5	14	4.6	
Day-care center 3 (n, %)	37	11.4	40	13.1	
Day-care center 4 (n, %)	28	8.6	22	7.2	
Day-care center 5 (n, %)	19	5.9	32	10.5	
Day-care center 6 (n, %)	28	8.6	34	11.2	
Day-care center 7 (n, %)	42	13.0	49	16.1	
Day-care center 8 (n, %)	35	10.8	18	5.9	
Day-care center 9 (n, %)	32	9.9	32	10.5	
Day-care center 10 (n, %)	25	7.7	45	14.8	
Presence of comorbidity (n, %)	69	21.3	54	17.7	0.256
Size of household (mean, SD)	3.68	0.85	3.91	1.04	0.002
Number of siblings (mean, SD)	1.66	0.71	1.94	0.82	<0.001
Number of smokers in home (mean, SD)	0.49	0.66	0.63	0.71	0.011

Table 2 Sex-adjusted HR and its 95%CI of trivalent inactive vaccine on influenza incidence in 2011–2012 season

Age	Vaccine group				No vaccine group						
	n	Person-days	Incidence	Incidence rate‡	*n*	Person-days	Incidence	Incidence rate‡	HR	95%CI	*P*-value
0 years	3	506	1	19.8	40	6 921	4	5.8	2.23	0.20, 24.60	0.513
1 year	75	12 973	8	6.2	47	7 194	17	23.6	0.24	0.10, 0.56	0.001
2 years	85	13 971	19	13.6	42	6 675	14	21.0	0.66	0.33, 1.33	0.246
3 years	61	9 847	17	17.3	58	8 443	26	30.8	0.56	0.31, 1.04	0.067
4 years	53	8 455	15	17.7	53	8 205	16	19.5	0.90	0.44, 1.83	0.760
5 or 6 years	47	7 519	12	16.0	65	10 791	14	13.0	1.23	0.57, 2.67	0.602
Total	324	53 271	72	13.5	305	48 229	91	18.9	0.71†	0.52, 0.97	0.032

Incidence was defined as being affected with influenza diagnosed by pediatrician. †Age- and sex-adjusted HR in the total subjects. ‡Incidence rate per 10 000 person-days. HR, hazard ratio.

decreased in the total subjects (HR = 0.71, 95%CI 0.52–0.97). As shown in Table 3, the HR of TIV on the influenza incidence were not meaningfully changed even after adjusting potential confounding variables, such as the day-care center, presence of comorbidity, size of household, number of siblings, and number of smokers in the home in addition to age and sex of the patient. Namely, HR was significantly reduced in the subjects aged 1 year (HR = 0.22, 95%CI 0.09–0.54) as well as in the total subjects (HR = 0.72, 95%CI 0.52–0.99). Consequently, effectiveness of TIV was calculated as 78% for the subjects aged 1 year, and 28% for the total subjects.

Discussion

It was found that the HR of TIV on influenza incidence was significantly reduced in the subjects aged 1 year and in the total subjects, but not in the subjects aged 0 years, or 2–6 years. Fujieda *et al*.[14] reported, from the results of a follow-up study at 54 pediatric clinics in eight areas of Japan in the 2002–2003 season, that risk was significantly reduced in the group, aged 2.0–3.9 years, receiving an inactivated vaccine, but not those aged under 1.9 years or over 4.0 years. Similar to this study, they found an insignificantly increased risk of an inactivated vaccine among children less than 1 year of age, and they mentioned that there was a lower immune response to the influenza vaccine for those less than 1 year of age.[14]

Maeda *et al*.[12] showed, with a prospective cohort study in Japan, that the risk of an influenza-like illness was insignificantly reduced in the group receiving the inactivated vaccine of age strata from 1 year to 7 years of age. Similar to this study's results, they found a significantly decreased risk of the inactivated vaccine on influenza infection in the total number of children aged 1–7 years. As explained by Hirota *et al*.,[19] the variety in results comes from the fact that efficacy or effectiveness of the vaccine is influenced by the designs or conditions in the fields, such as a mixed epidemic with different strains, antigenic similarity between the vaccine strains and epidemic viruses, and inter-individual variation in the antibody response to the vaccine.

Table 3 HR and its 95%CI of trivalent inactive vaccine on influenza incidence in 2011–2012 season, after adjusting potential confounding variables†

Age	HR	95%CI	*P*-value
0 years	2.47	0.08, 73.63	0.602
1 year	0.22	0.09, 0.54	0.001
2 years	0.60	0.28, 1.28	0.185
3 years	0.66	0.35, 1.27	0.215
4 years	0.75	0.36, 1.54	0.427
5 or 6 years	1.37	0.62, 3.04	0.438
Total	0.72	0.52, 0.99	0.042

†Distribution of day-care center, presence of comorbidity, size of household, number of siblings, and number of smokers in home, were adjusted in addition to sex and age. HR, hazard ratio.

The efficacy of the influenza vaccine has been reported to be higher in fully vaccinated children with two doses than in partially vaccinated children with one dose.[20,21] However, Gruber *et al*.[10] showed that a single dose of TIV produced a sufficient serologic rise to influenza viral antigen, and might protect against viral infection. It should be mentioned that the research by Gruber *et al*.[10] was performed among school-age children, and immunological backgrounds may be different from pre-school children. Because a majority of the vaccinated subjects (93.2%) were fully vaccinated with two doses of TIV, it was not possible to compare the effectiveness between one and two doses in this study.

The influenza incidence was defined as that diagnosed by a pediatrician, although information was not obtained about either cultural confirmation or the subtype of influenza. A report about the sampling study on the cultural confirmation of suspected specimen from clinics in Sapporo City showed that 91.4% of them were the influenza virus.[18] Furthermore, according to surveillance by Sapporo City Hygiene Research Center,[22] endemic of the influenza virus A/H3N2 was observed from the 51st week of 2011 to the 14th week of 2012, and its peak was at the 4th week of 2012. In addition, the spread of the influenza virus B was observed from the 3rd week of 2012 to the 20th week of 2012. The proportion of patients with influenza was reported to be about 71% in influenza A/H3N2 and about 28% in influenza B in the entire 2011–2012 season. We considered that the endemic of influenza in the study population was consistent with endemic of influenza in the entire Sapporo City. In addition, it was reported that the antigenicity of 2011–2012 endemic influenza A (H3N2) and B strains were concordant with those of 2011–2012 vaccine strains in around 60% and 70%, respectively (IASR 33: 288–294, 2012).

Although the amount of influenza vaccine given to children increased in the 2011–2012 season from 0.1 mL to 0.25 mL for those aged 0 years, from 0.2 mL to 0.25 mL for those aged 1–2 years, and from 0.2 mL to 0.5 mL for those aged 3–5 years, it was not possible for us to evaluate the effect of these increments, because the appropriate comparative population could not be obtained. Although we set the initial date of observation at 1 October 2011, the initial date of observation for each subject with or without vaccination is controversial for analysis with the Cox model. Therefore, we applied analysis by the logistic regression model in addition to analysis by the Cox model. As a result, we could obtain the similar risk estimates in association of influenza vaccination with influenza infection between these two analyses (the odds ratios obtained with the logistic regression analysis are not shown in this article).

As a limitation of this study, only 40% of study candidates responded to the request to participate in this study. Accordingly, a selection bias might exist in this study. Ideally the incidence of influenza should be confirmed by observing protocols at every medical institution, or observing records of high fever in every day-care center. However, it was not practical for us to access medical records at all medical institutions or records of high fever at the day-care centers. It was thought that distribution of the day-care centers, size of household, number of siblings, and the number of smokers in home were all potential confounding factors in the association between vaccination and influenza incidence. Especially, different status of influenza endemic was observed in 10 day-care centers as shown in Table 1, and one day-care center showed a significantly increased risk of influenza infection (HR = 2.53, 95%CI 1.48–4.34). However, it was not the case in this study, because HR of TIV on the influenza incidence were not altered even after adjusting all of them, as shown in Table 3.

In conclusion, HR of TIV on the influenza incidence was significantly reduced in the subjects aged 1 year and in the total subjects, but not in the subjects aged 0 years, or 2–6 years. Further studies are necessary in different seasons, places, and populations to clarify the effectiveness of the influenza vaccine in children.

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References

1 Togashi T, Matsuzono Y, Narita M. Epidemiology of influenza-associated encephalitis-encephalopathy in Hokkaido, the northernmost island, Japan. *Pediatr. Int.* 2000; **42**: 192–6.
2 Nishimura N. Seasonal influenza. *Rinsho Kensa* 2010; **54**: 1334–8 (Japanese text with English abstract).
3 Weinberg GA, Szilagyi PG. Vaccine epidemiology: efficacy, effectiveness, and the translational research roadmap. *J. Infect. Dis.* 2010; **201**: 1607–10.
4 Osterholm MT, Kelley N, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. *Lancet Infect. Dis.* 2012; **12**: 36–44.
5 Belshe RB, Mendelman PM, Treanor J *et al*. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children. *N. Engl. J. Med.* 1998; **338**: 1405–12.
6 Belshe RB, Gruber WC, Mendelman PM *et al*. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. *J. Pediatr.* 2000; **136**: 168–75.
7 Vesikari T, Fleming DM, Aristegui JF *et al*. Safety, efficacy, and effectiveness of cold-adapted influenza vaccine-trivalent against community-acquired, culture-confirmed influenza in young children attending day care. *Pediatrics* 2006; **118**: 2298–312.
8 Tam JS, Capeding MRZ, Lum LCS *et al*. Efficacy and safety of a live attenuated, cold-adapted influenza vaccine, trivalent against culture-confirmed influenza in young children in Asia. *Pediatr. Infect. Dis. J.* 2007; **26**: 619–28.
9 Neto HB, Farhat CK, Tregnaghi MW *et al*. Efficacy and safety of 1 and 2 doses of live attenuated influenza vaccine in vaccine-naïve children. *Pediatr. Infect. Dis. J.* 2009; **28**: 365–71.
10 Gruber WC, Taber LH, Glezen WP *et al*. Live attenuated and inactivated influenza vaccine in school-age children. *Am. J. Dis. Child.* 1990; **144**: 595–600.
11 Clover RD, Crawford S, Glezen WP, Taber LH, Matson CC, Couch RB. Comparison of heterotypic protection against influenza A/Taiwan/86 (H1N1) by attenuated and inactive vaccine to A/Chile/83-like viruses. *J. Infect. Dis.* 1991; **163**: 300–4.
12 Maeda T, Shintani Y, Miyamoto H *et al*. Prophylactic effect of inactivated influenza vaccine on young children. *Pediatr. Int.* 2002; **44**: 43–6.
13 Kawai N, Ikematsu H, Iwaki N *et al*. A prospective, internet-based study of the effectiveness and safety of influenza vaccination in the 2001–2002 influenza season. *Vaccine* 2003; **21**: 4507–13.
14 Fujieda M, Maeda A, Kondo K, Kaji M, Hirota Y. Inactivated influenza vaccine effectiveness in children under 6 years of age during the 2002–2003 season. *Vaccine* 2006; **24**: 957–63.
15 Belshe RB, Edwards KM, Vesikari T *et al*. Live attenuated versus inactivated influenza vaccine in infants and young children. *N. Engl. J. Med.* 2007; **356**: 685–94.
16 Neuzil KM, Dupont WD, Wright PF, Edwards KM. Efficacy of inactivated and cold-adapted vaccines against influenza A infection, 1985 to 1990: the pediatric experience. *Pediatr. Infect. Dis. J.* 2001; **20**: 733–40.
17 Khan AS, Polezhaev F, Vasiljeva R *et al*. Comparison of US inactivated split-virus and Russian live attenuated, cold-adapted trivalent influenza vaccines in Russian schoolchildren. *J. Infect. Dis.* 1996; **173**: 453–6.
18 Kikuchi M, Sato N, Ouguiya Y, Ito H, Takahashi H, Sasaki Y. Endemic of influenza in 2011–2012 season in Sapporo City and surveillance on Osemitamibil resistant subtype. *Annu. Rep. Sapporo City Hyg. Res. Center* 2012; **39**: 79–83 (Japanese text).
19 Hirota Y, Kaji M, Ide S *et al*. Antibody efficacy as a keen index to evaluate influenza vaccine effectiveness. *Vaccine* 1997; **15**: 962–7.
20 Ritzwoller DP, Bridges CB, Shetterly S, Yamasaki K, Kolczak M, France EK. Effectiveness of the 2003–2004 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. *Pediatrics* 2005; **116**: 153–9.
21 Allison MA, Daley MF, Crane LA *et al*. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003–2004 season. *J. Pediatr.* 2006; **149**: 755–62.
22 Sapporo City Hygiene Research Center. Detection of influenza virus. 2012. [Cited 16 July 2013.] Available from URL: http://www.city.sapporo.jp/eiken/infect/detect/graph/2011c501.html

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Immunogenicity of the Trivalent Inactivated Influenza Vaccine in Young Children Less than 4 Years of Age, with a Focus on Age and Baseline Antibodies

Ayumi Mugitani,[a] Kazuya Ito,[a] Shin Irie,[b] Takashi Eto,[c] Motoki Ishibashi,[c] Satoko Ohfuji,[d] Wakaba Fukushima,[d] Akiko Maeda,[d] Yoshio Hirota,[e,f] for the Fukuoka Pediatricians Group for Vaccine Efficacy

Medical Co. LTA Sumida Hospital, Tokyo, Japan[a]; Medical Co. LTA, Fukuoka, Japan[b]; Medical Co. LTA PS Clinic, Fukuoka, Japan[c]; Department of Public Health, Osaka City University Graduate School of Medicine, Osaka, Japan[d]; Medical Co. LTA Clinical Epidemiology Research Center, Fukuoka, Japan[e]; Osaka City University, Osaka, Japan[f]

In this study, we assessed the effects of the prevaccination titer and age on the immunogenicity of a low dose of influenza vaccine in children less than 4 years of age. A total of 259 children received two vaccine doses (0.1 ml for 0-year-olds and 0.2 ml for children 1 year of age or older) 4 weeks apart during the 2005/2006 season. The hemagglutination inhibition antibody titers were measured before vaccination and 4 weeks after the first and second doses. The geometric mean titer, mean fold rise, seroresponse proportion (≥4-fold rise in titer), and seroprotection proportion (titer ≥1:40) were calculated for the prevaccination titer and age categories. A multivariate logistic regression analysis was performed using the seroresponse and seroprotection proportions as dependent variables and the prevaccination titer and age as explanatory variables. As for the seroresponse against the H1 antigen after the first dose, the adjusted odds ratios of the prevaccination titers (versus <1:10) were 2.2 (95% confidence interval, 0.8 to 5.8) at 1:10 to 1:20 and 0.14 (0.04 to 0.49) at ≥1:40. The corresponding figures for ages were 0.03 (0.01 to 0.07) for the 0-year-olds and 0.17 (0.08 to 0.34) for the 1-year-olds compared with the 2- to 3-year-olds ($P_{trend} < 0.001$). Similar results were also obtained for the H3 and B strains. Significantly elevated odds ratios for seroprotection were observed with greater prevaccination titers and older ages for all strains. The prevaccination titer and age were independently associated with the antibody response in young children. The immune response was weaker in the younger children and those without preexisting immunity.

Influenza is a vaccine-preventable disease. The rate of seasonal influenza infection is highest among children, and children less than 2 years of age are at high risk of influenza-associated hospitalization (1, 2). The Advisory Committee on Immunization Practices routinely recommends that children 6 months to 8 years of age receive two doses of influenza vaccine during their first season of vaccination in order to optimize the immune response (3). This recommendation is based on data showing that vaccine effectiveness and immunogenicity are lower among young children treated with one dose of the vaccine, whereas two doses of vaccine provide substantial protection against influenza-like illness (ILI) (4–6) and induce a protective level of antibodies, even in young children (7–15).

The factors affecting low immune responses to the influenza vaccine among children are supposed to include immature immunity function due to age, infrequency of opportunity for exposure to influenza virus through vaccination and/or infection, thus resulting in a lack of induced priming, and a low-volume dose of the vaccine. As the subjects get older, it has been reported that their prevaccination titer (pretiter) increases (16–19), but there has been very little detailed research that considered the predictive factors in the immune response (20, 21).

In this report, we present the immunogenicity of the trivalent inactivated influenza vaccine (IIV3) in young children. More specifically, by using a thorough descriptive analysis and multivariate analysis, we performed a detailed evaluation of our preliminary 2005/2006 data (22), focusing on the mutual effects of age and the pretiter status, which are considered to be essential for evaluating the immunogenicity of young children.

MATERIALS AND METHODS

Study subjects and vaccination. Healthy infants and children 6 months to 3 years of age were eligible for enrollment. The children were recruited from six pediatric practices in Japan. The exclusion criteria were fever or acute serious illness at the time of vaccination, a history of anaphylaxis to the vaccine components, and/or other conditions that rendered the subjects ineligible to receive vaccination. We attempted to register approximately 50 children in each age group (0-, 1-, 2-, and 3-year-olds); a total of 259 children were enrolled. The study protocol was approved by the ethics committee of the Osaka City University Faculty of Medicine, and written informed consent was obtained from the guardians of all children.

A single lot of licensed trivalent inactivated, thimerosal-free, unadjuvanted influenza HA vaccine (FLUBIK HA, lot HE01A; Biken, Japan) was used in this study. Each vaccine contained 15 μg/0.5 ml of each of the three hemagglutinin antigens recommended for the 2005/2006 influenza vaccine: the A/New Caledonia/20/99 (H1N1), A/NewYork/55/2004 (H3N2), and B/Shanghai/361/2002 strains.

The subjects received two subcutaneous injections of IIV3 in the arm, at a dose of 0.1 ml for 0-year-olds and 0.2 ml for children ≥1 year of age, in conformity with the Japanese influenza vaccine regulations at that time. All subjects received the first vaccine dose between 1 September and 31

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Address correspondence to Ayumi Mugitani, a.mugitani@gmail.com.

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October 2005, followed by the second dose 4 weeks later between 1 October and 31 November 2005. None of the children experienced physician-diagnosed influenza virus infection between the first and second dose or discontinued participation due to an adverse event and/or experienced any severe adverse events. Hence, all subjects were included in the analyses. According to the national infectious diseases surveillance, the 2005/2006 seasonal epidemic occurred in mid-December. This was at least 2 weeks after all children had received their second vaccination.

Information collection and antibody titer measurement. The following information was collected via a self-administered questionnaire completed by the guardian: baseline characteristics, such as age, sex, body weight, and underlying medical conditions; previous influenza vaccination status within the past 3 years; and a history of ILI with a fever of ≥39°C during the last season.

A triplet serum sample was obtained before vaccination (S0), 4 weeks after the first dose (S1), i.e., immediately before the second dose, and 4 weeks after the second dose (S2). The sera were stored frozen at −70°C to −80°C until they were analyzed simultaneously. The hemagglutination inhibition (HI) antibody titers for each vaccine antigen were measured according to a standard assay using type O human erythrocytes (23).

Statistical analyses. The outcome measurements of this study, which aimed to assess immunogenicity, were the geometric mean titer (GMT), mean fold rise (MFR), proportion of subjects with a ≥4-fold rise in the postvaccination titer (sR), and proportion of subjects achieving a titer of ≥1:40 (sP). For data processing, a titer of <1:10 was assigned a value of 1:5, and reciprocal antibody titers were handled after logarithmic transformation. Therefore, the use of 1:5 titers for lower censored values may lead to a reduced estimate of the variance. The results are presented in the original scale by calculating the antilogarithms. The data were categorized to examine the effects of the following factors considered to be medically important based on previous reports: pretiter (<1:10, 1:10 to 1:20, and ≥1:40), age, influenza vaccination within the past 3 years, and ILI history during the last season.

The significance of the MFR within a category was assessed according to the Wilcoxon signed-rank test, while intercategory comparisons of GMT and MFR values were made using either the Wilcoxon rank sum test or the Kruskal-Wallis test. The *t* test, analysis of variance, Mantel-extension method for trend test, and χ^2 test were also employed where appropriate.

The independent effects of the pretiter status and age on antibody induction were evaluated using a multivariate logistic regression analysis. The models were constructed with sR or sP as a dependent variable and the pretiter status and age as explanatory variables. The odds ratios (ORs) and the 95% confidence intervals (CIs) are presented. The influenza vaccination history and ILI history were excluded from the final model after consideration of the correlations between these factors and age. In addition, if both factors were included together, we would have been forced to exclude 0-year-old infants who mostly did not have a vaccination history or ILI history (100% and 89%, respectively) from the analysis. This results in exclusion of children with a pretiter of <1:10, accounting for the majority of the subjects, and thus the validity of the multivariate analysis itself would have been compromised. Therefore, we excluded these parameters from the analysis to secure a sufficient number of subjects. A *P* value of <0.05 was considered to be statistically significant. All hypothesis tests were two-sided. The calculations were performed using the SAS version 9.2 software program (SAS Institute Inc., Cary, NC).

RESULTS

The baseline characteristics of the subjects are shown in Table 1. The mean and median ages were nearly the same (24.1 and 24.0 months). The subjects were distributed almost equally (64 to 66 subjects) among the four age groups. Asthma, urticaria, and atopic dermatitis were relatively frequent underlying diseases (5.0% to 6.6%).

TABLE 1 Characteristics of study subjects

Variable	Value[a]
Total no. of subjects	259
Male sex	142 (54.5)
Age at vaccination (mo)	
Mean (SD)	24.1 (12.4)
Median (range)	24.0 (45.0)
Age at vaccination	
0 yr	64 (25)
1 yr	65 (25)
2 yr	64 (25)
3 yr	66 (25)
Underlying illnesses	
Heart disease	1 (0.4)
Renal disease	1 (0.4)
Anemia	2 (0.8)
Asthma	14 (5.4)
Urticaria	17 (6.6)
Atopic dermatitis	13 (5.0)
Influenza vaccination within the past 3 yr	
Vaccinated	114 (44)
0 yr	0 (0)
1 yr	17 (15)
2 yr	46 (40)
3 yr	51 (45)
Not vaccinated	144 (56)
0 yr	64 (44)
1 yr	48 (33)
2 yr	17 (12)
3 yr	15 (10)
Influenza-like illness during the last season present	122 (47)

[a] Values are expressed as no. (%) unless otherwise indicated.

Geometric mean titer and mean fold rise. The GMT and MFR values in the subjects grouped according to the pretiter status, age, influenza vaccination history, and ILI history are summarized in Table 2 for each antigen. Approximately three-fourths of the children fell into the seronegative category (pretiter of <1:10), regardless of the type of test antigen (77%, 72%, and 73% for H1, H3, and B, respectively). The proportion of children with a pretiter of ≥1:40 was highest for the H3 antigen (24%) followed by the B (12%) and H1 (6%) antigens.

A higher pretiter against the H1 antigen was associated with a higher mean age and greater pre- and postvaccination GMT values (S0, S1, and S2) ($P < 0.05$ for each by analysis of variance [ANOVA] or the Kruskal-Wallis rank test). The MFR after the first dose (S1/S0) was higher in the 1:10 to 1:20 category (5.7-fold) than those in the <1:10 and ≥1:40 categories (3.0- and 2.3-fold, respectively). The S2/S1 values further increased 2.4-fold in the pretiter of <1:10 category, but not in the two higher pretiter categories (1.1-fold in both). After the second dose (S2/S0), a ≥6-fold rise was seen in the <1:10 and 1:10 to 1:20 categories compared to that in the ≥1:40 category (2.6-fold). Therefore, the subjects with a pretiter of ≥1:40 showed lower MFR values at both S1 and S2. The trends for GMT and MFR were similar for the H3 and B antigens, with substantially pronounced changes in H3. The prevaccination GMT against H3 was quite high in the ≥1:40 category (208 at S0), leading to far more elevated postvaccination

TABLE 2 Geometric mean and mean fold rise

Vaccine antigen and category	No. (%) of subjects	Mean age (yr)	GMT[a]			MFR[b]		
			S0	S1	S2	S1/S0	S2/S1	S2/S0
A/New Caledonia/20/99(H1N1)								
Entire sample	259 (100)	1.5	7	23	46	3.3	2.0	6.6
Prevaccination titer								
<1:10	200 (77)	1.2[c]	5[d]	15[d]	36[d]	3.0[d,e]	2.4[d,e]	7.1[d,e]
1:10–1:20	44 (17)	2.4	15	84	94	5.7[e]	1.1	6.4[e]
≥1:40	15 (6)	2.6	66	153	176	2.3[e]	1.1	2.6[e]
Age								
0 yr	64 (25)	0.7[c]	5[d]	6[d]	21[d]	1.2[d,e]	3.3[d,e]	4.0[d,e]
1 yr	65 (25)	1.4	5	14	39	2.7[e]	2.7[e]	7.3[e]
2 yr	64 (25)	2.4	8	58	80	7.4[e]	1.4[e]	10.3[e]
3 yr	66 (25)	3.4	11	54	70	5.0[e]	1.3[e]	6.4[e]
Influenza vaccination in the past 3 yr								
Unvaccinated	144 (56)	0.9[c]	5[d]	9[d]	32[d]	1.8[d,e]	3.5[d,e]	6.1[e]
Vaccinated	114 (44)	2.3	10	73	72	7.4[e]	1.0	7.3[e]
Influenza-like illness during the last season								
Absent	136 (53)	1.2[c]	7	16[d]	37[d]	2.2[d,e]	2.3[d,e]	5.2[d,e]
Present	122 (47)	1.9	7	35	59	5.1[e]	1.7[e]	8.7[e]
A/New York/55/2004(H3N2)								
Entire sample	259 (100)		13	37	71	2.8	2.0	5.5
Prevaccination titer								
<1:10	187 (72)	1.2[c]	5[d]	12[d]	29[d]	2.3[d,e]	2.5[d,e]	5.9[d,e]
1:10–1:20	9 (4)	2.1	15	235	296	16.0	1.3	20.2
≥1:40	63 (24)	2.3	208	852	806	4.1[e]	0.9	3.9[e]
Age								
0 yr			6[d]	8[d]	32[d]	1.4[d,e]	4.1[d,e]	5.5[e]
1 yr			8	20	51	2.4[e]	2.5[e]	6.1[e]
2 yr			22	108	130	5.0[e]	1.2[e]	6.0[e]
3 yr			27	105	123	4.0[e]	1.2[e]	4.6[e]
Influenza vaccination in the past 3 yr								
Unvaccinated			9[d]	17[d]	53[d]	1.9[d,e]	3.1[d,e]	5.9[e]
Vaccinated			20	97	105	4.8[e]	1.1	5.2[e]
Influenza-like illness during the last season								
Absent			10[d]	23[d]	54[d]	2.4[d,e]	2.3[d,e]	5.6[e]
Present			18	61	96	3.5[e]	1.6[e]	5.5[e]
B/Shanghai/361/2002								
Entire sample	259 (100)		8	22	34	2.8	1.6	4.4
Prevaccination titer								
<1:10	188 (73)	1.3[c]	5[d]	10[d]	19[d]	2.0[d,e]	1.9[d,e]	3.7[d,e]
1:10–1:20	40 (15)	2.2	13	126	121	9.5[e]	1.0	9.2[e]
≥1:40	31 (12)	2.2	65	274	274	4.2[e]	1.0	4.2[e]
Age								
0 yr			5[d]	6[d]	13[d]	1.1[d]	2.3[d,e]	2.5[d,e]
1 yr			7	20	32	2.7[e]	1.6[e]	4.4[e]
2 yr			8	40	52	4.9[e]	1.3[e]	6.3[e]
3 yr			12	50	62	4.2[e]	1.2	5.1[e]
Influenza vaccination in the past 3 yr								
Unvaccinated			6[d]	11[d]	23[d]	1.7[d,e]	2.2[d,e]	3.8[d,e]
Vaccinated			11	55	56	5.1[e]	1.0	5.2[e]
Influenza-like illness during the last season								
Absent			7[d]	14[d]	24[d]	2.0[d,e]	1.8[d,e]	3.6[d,e]
Present			9	38	51	4.1[e]	1.3[e]	5.5[e]

[a] GMT, geometric mean titer.
[b] MFR, mean fold rise.
[c] $P < 0.05$ by *t* test or ANOVA.
[d] $P < 0.05$ by the Wilcoxon rank sum test or Kruskal-Wallis rank test for intercategory comparisons.
[e] $P < 0.05$ by the Wilcoxon signed-rank test for intracategory comparisons.

GMT values (852 at S1 and 806 at S2). In addition, the GMT values in the 1:10 to 1:20 category also increased greatly after the first dose (235 at S1; S1/S0 = 16.0-fold).

When the data were examined according to age group, the pre- and postvaccination GMT values against H1 increased with increasing age ($P < 0.05$ at each time point for the Kruskal-Wallis rank test). A similar tendency was seen in the MFR S1/S0 and S2/S0 values ($P < 0.05$ at both time points for the Kruskal-Wallis rank test), with maximum values in the 2-year-olds (7.4- and 10.3-fold, respectively). An opposite trend was observed in the S2/S1 values, i.e., the MFR decreased with increasing age ($P < 0.05$ for the Kruskal-Wallis rank test). Comparable findings regarding GMT and MFR were also obtained for H3 and B, with distinctively elevated postvaccination GMT values against H3 in the older age groups. The pre- and postvaccination GMT values were consistently higher in the children with a history of vaccination or ILI than in those with no such history, at all time points for every strain.

The above findings can be summarized as follows. (i) Approximately 70% of the children were initially seronegative (pretiter of <1:10). (ii) A higher pretiter and older age were associated with elevated GMT values after the first and second doses, irrespective of the test antigen. (iii) The maximum titers varied with the antigens: the highest GMT value (exceeding 1:800) was attained for the high pretiter category against H3. (iv) In the pretiter of <1:10 category, the response after the first dose was weak, and an additional titer was induced by the second dose. On the other hand, in the pretiter of ≥1:10 category, the titer reached a plateau after the first dose, and no or little booster response was induced after the second dose. (v) The MFR values were lower after both the first and second doses in the pretiter of ≥1:40 category and the 3-year-olds.

Seroresponse proportion and seroprotection proportion. The top section of Fig. 1 shows the sR for each antigen. Comparing the three levels of the pretiter, the sR for <1:10 against H1 increased from 45% after the first dose (S1/S0) to 77% after the second dose (S2/S0). The corresponding values were 34% to 73% for H3 and 31% to 54% for B. In the 1:10 to 1:20 category, the sR reached nearly 90% (85% to 89%) with the first dose alone for all antigens. However, in the ≥1:40 category, the sR after one dose did not exhibit a large increase (33 to 62%) and instead reached a plateau even after the second dose (47 to 60%). An analysis of the sP (the bottom section of Fig. 1) was performed, excluding children with a pretiter of ≥1:40. The sP in the pretiter of <1:10 category was low, even with two doses (37 to 58%), whereas in the 1:10 to 1:20 category, more than about 90% of the subjects attained a seroprotective titer (≥1:40) with one dose alone.

Next, stratified analyses were conducted to examine the effects of the pretiter and age (Fig. 2 and 3). The age-specific sR and sP values were calculated after stratification for the three levels of the pretiter. Among those with a pretiter of <1:10, the S1/S0 sR was considerably lower in the younger children (0 years, 3 to 10%; 1 year, 20 to 36%) than in the 2- to 3-year-olds (61 to 82%) for each antigen, indicating an increase in sR with increasing age ($P < 0.001$ for each in the Mantel-extension method for trend test). The S2/S0 sR further increased in all age groups, maintaining a dose-response relationship similar to that observed for the S1/S0 sR. On the other hand, in the pretiter 1:10 to 1:20 group, all of the 1-year-olds achieved the sR level with one dose alone (100%), and slightly lower sR values were seen in the 2- to 3-year-olds (85 to 87%).

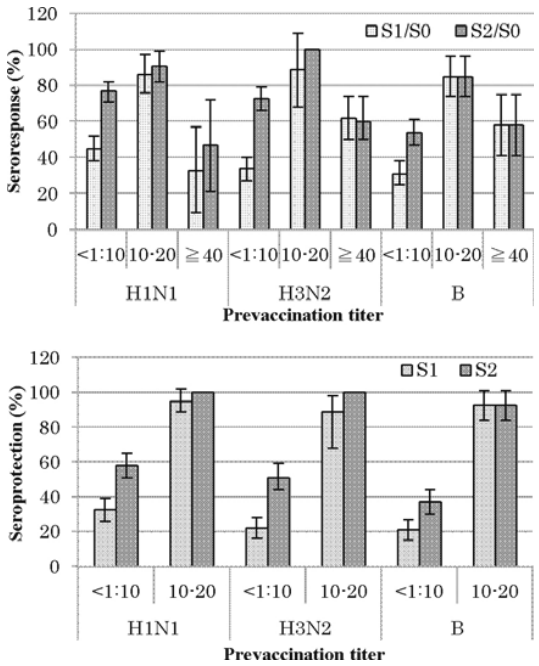

FIG 1 Seroresponse (≥4-fold rise) and seroprotection (HI titer of ≥1:40) proportion and 95% CIs. Subjects with prevaccination titers of ≥1:40 were excluded for the seroprotection analyses. S0, before vaccination. S1, after the first dose; S2, after the second dose.

However, in the pretiter ≥1:40 group, the S1/S0 sR for H3 decreased with increasing age ($P < 0.03$ in the Mantel-extension method for trend test), and a similar tendency was seen against H1 and B, although the skewed distribution of the subjects made it difficult to statistically confirm this finding. In addition, in the pretiter ≥1:40 group, no or little booster response to the second dose was induced for any antigen in any age category.

The sP showed the same trend as the sR. In the pretiter of <1:10 group, the sP values improved with age for all antigens, and an additional antibody titer was induced by the second dose, although the sP value against H1 at S2 was at most 78% in the 2- to 3-year-olds. In contrast, in the 1:10 to 1:20 group, a substantial rise in titer was achieved with one dose alone, irrespective of the age category for all antigens, except in the 0-year-olds for B (67%).

Therefore, both the pretiter and age *per se* may mutually influence the antibody response. Hence, the independent effects of the pretiter and age on sR and sP were examined using a logistic regression model (Table 3).

The crude ORs (95% CIs) of the pretiter for sR after the first dose were significantly high at 1:10 to 1:20 (versus <1:10): 7.7 (3.1 to 19.1) for H1, 15.8 (1.9 to 129) for H3, and 12.4 (4.9 to 31.1) for B. These values shifted toward null when the effect of age was simultaneously considered: the adjusted ORs (95% CIs) were 8.8 (1.1 to 73.1) for H3 and 9.2 (3.2 to 26.6) for B, with statistical significance. However, the pretiter of ≥1:40 category demonstrated lower ORs for all test antigens than the pretiter of 1:10 to 1:20 category in both the univariate and multivariate analyses. For sP, significantly elevated ORs were observed in the pretiter of 1:10 to 1:20 category, although this trend was unstable, as indicated by the wide CIs. The adjusted ORs (95% CIs) were 5.4 (2.1 to 13.6) for H1, 16.3 (1.8 to 148) for H3, and 47.5 (11.5 to 197) for B.

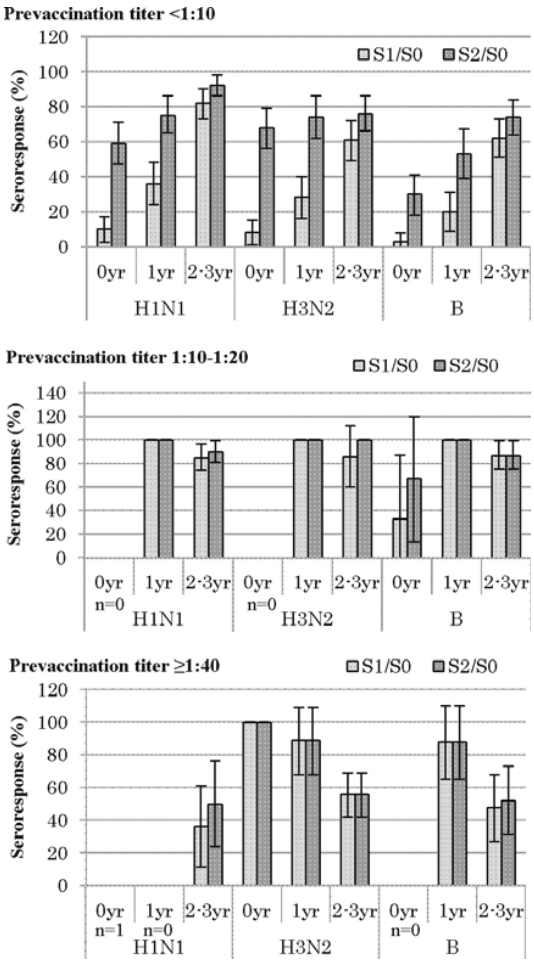

FIG 2 Age-specific seroresponse proportion and 95% CIs stratified by prevaccination titer. S0, before vaccination; S1, after the first dose; S2, after the second dose; n, total no. of subjects.

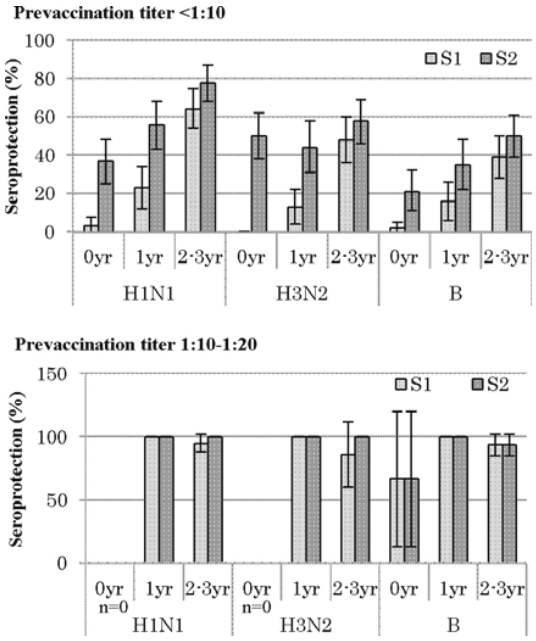

FIG 3 Age-specific seroprotection proportion and 95% CIs stratified by prevaccination titer. Subjects with prevaccination titers of ≥1:40 were excluded from the seroprotection analyses. S0, before vaccination; S1, after the first dose; S2, after the second dose; n, total no. of subjects.

Age was found to be positively associated with sR and sP for all antigens. Against H1, the adjusted ORs (95% CIs) for sR after the first dose were 0.17 (0.08 to 0.34) in the 1-year-olds and 0.03 (0.01 to 0.07) in the 0-year-olds, with a clear dose-response relationship, compared with the 2- to 3-year-olds as the reference ($P_{trend} < 0.001$). Similar results were obtained for H3 and B ($P_{trend} < 0.001$ for both). In addition, significantly elevated ORs for sP after the first dose were observed in association with an older age for all strains ($P_{trend} < 0.001$ for H1 and H3). For both sR and sP, the adjusted ORs were quite similar to the crude values, irrespective of the type of antigen, which suggests a low combined effect of age with the pretiter.

DISCUSSION

Previous research has proven that, in many cases, the immune response to the influenza vaccine is low among children. Although most of this research indicates the extent of the immune response, very few studies have delved into the predictive factors regarding this observation. In this study, we considered the response to the HI antibody, an immune correlate of protection against influenza infection induced after vaccination, along with the predictive factors thereof in young children up to 4 years of age.

Regarding seronegative children (pretiter of <1:10), a trend was noticed wherein the antibody titer rose postvaccination in line with age, but at the same time, regardless of the strain of vaccine, the immune response was low. A booster effect was obtained by vaccination twice, but the sP reached ≥70% only for the H1 antigen, which is one of the international criteria for approval (24, 25), following the second vaccination, and this was only achieved in the oldest children. These results were inconsistent with those of previous studies, which indicated that even young children can reach protective levels of antibody values if the vaccination is carried out twice (7–15). We think the reason that in this study, the vaccination dose used was smaller than the regulated volume used in Europe and the United States. The regulated IIV3 vaccination dose in Japan was lower than that used in Europe and the United States up to the 2010/2011 season (Japan: 0.1 ml for 0-year-olds, 0.2 ml for 1- to 5-year-olds, 0.3 ml for 6- to 12-year-olds, and 0.5 ml for 13-year-olds and older; Europe and the United States: 0.25 ml for children of 6 to 36 months of age and 0.5 ml for those older than 36 months). Although these dosage levels have been widely discussed for many years, they were maintained during the H1N1pdm09 pandemic. It was therefore assumed that if the current group were vaccinated twice with the high vaccine doses, even seronegative children with no antibody prior to vaccination would acquire an antibody titer that would cover them.

That said, seropositive children (pretiter of 1:10 to 1:20) demonstrated an excellent immune response, regardless of the strain of vaccine. In this group, minimum values of 85% sR and sP were achieved in children aged 1 year or older who were vaccinated with all strains with one dose alone. Children exposed to the influenza virus prior to vaccination, due either to an earlier vaccination or to

TABLE 3 Odds ratios for seroresponse and seroprotection proportions after the first vaccination[f]

Vaccine antigen and category	Seroresponse (S1/S0 ≥ 4)				Seroprotection (S1 ≥ 1:40)			
	Total no. of subjects	No. (%) with seroresponse	OR (95% CI)[a]		Total no. of subjects	No. (%) with seroprotection	OR (95% CI)[a]	
			Crude	Adjusted[b]			Crude	Adjusted[b]
A/New Caledonia/20/99(H1N1)								
Prevaccination titer								
<1:10	200	90 (45)	1.0	1.0	200	65 (33)	1.0	1.0
1:10–1:20	44	38 (86)	7.7 (3.1, 19.1)	2.2 (0.8, 5.8)	44	42 (95)	43.6 (10.2, 186)	5.4 (2.1, 13.6)
≥1:40	15	5 (33)	0.6 (0.2, 1.9)	0.14 (0.04, 0.49)				
P_{trend}			—[c]					
Age								
0 yr	64	6 (9)	0.01 (0.01, 0.08)	0.03 (0.01, 0.07)	63	2 (3)	0.01 (0.003, 0.05)	0.03 (0.01, 0.1)
1 yr	65	26 (40)	0.2 (0.1, 0.4)	0.17 (0.08, 0.34)	65	18 (28)	0.13 (0.06, 0.25)	0.2 (0.1, 0.4)
2–3 yr	130	101 (78)	1.0	1.0	116	87 (75)	1.0	1.0
P_{trend}			—[d]	—[d]			—[d]	—[d]
A/New York/55/2004 (H3N2)								
Prevaccination titer								
<1:10	187	63 (34)	1.0	1.0	187	41 (22)	1.0	1.0
1:10–1:20	9	8 (89)	15.8 (1.9, 129)	8.8 (1.1, 73.1)	9	8 (89)	28.5 (3.5, 234)	16.3 (1.8, 148)
≥1:40	63	39 (62)	3.2 (1.8, 5.8)	1.7 (0.9, 3.3)				
P_{trend}			—[d]					
Age								
0 yr	64	7 (11)	0.08 (0.04, 0.19)	0.11 (0.04, 0.26)	62	0	NA	NA
1 yr	65	29 (45)	0.4 (0.2, 0.8)	0.5 (0.3, 0.9)	56	9 (16)	0.18 (0.08, 0.42)	0.18 (0.08, 0.44)
2–3 yr	130	78 (60)	1.0	1.0	78	40 (51)	1.0	1.0
P_{trend}			—[d]	—[d]				
B/Shanghai/361/2002								
Prevaccination titer								
<1:10	188	59 (31)	1.0	1.0	188	39 (21)	1.0	1.0
1:10–1:20	40	34 (85)	12.4 (4.9, 31.1)	9.2 (3.2, 26.6)	40	37 (93)	47.1 (13.8, 161)	47.5 (11.5, 197)
≥1:40	31	18 (58)	3.0 (1.4, 6.6)	1.5 (0.7, 3.4)				
P_{trend}			—[d]	—[e]				
Age								
0 yr	64	3 (5)	0.03 (0.01, 0.09)	0.03 (0.01, 0.11)	64	3 (5)	0.04 (0.01, 0.14)	0.04 (0.01, 0.17)
1 yr	65	23 (35)	0.3 (0.2, 0.5)	0.4 (0.2, 0.7)	65	23 (35)	0.3 (0.1, 0.5)	0.3 (0.1, 0.8)
2–3 yr	130	85 (65)	1.0	1.0	130	85 (65)	1.0	1.0
P_{trend}			—[d]	—[d]			—[d]	—[d]

[a] OR, odds ratio; CI, confidence interval.
[b] Adjusted for prevaccination titer and age.
[c] $P < 0.05$.
[d] $P < 0.001$.
[e] $P < 0.01$.
[f] Subjects with prevaccination titer of ≥1:40 were excluded from the seroprotection analyses.

becoming infected with influenza, who retained a certain level of antibodies have been demonstrated as being capable of achieving a sufficient antibody value with only a single vaccination, even at a young age. These results support the current recommendation that children aged between 6 months and 8 years who have completed a course of two vaccinations in the previous year require only one vaccination in the following season.

Children with a minimum pretiter of 1:40, however, regardless of the strain of vaccine, demonstrated a slightly lower sR than seropositive children, a tendency which was particularly noticeable in the older group (the 2- to 3-year-olds). Furthermore, no booster effect was apparent upon a second vaccination. A phenomenon in which the antibody titer reaches a certain level but then plateaus (negative feedback) has been reported, suggesting a risk that if the immune response is evaluated without stratification of the pretiter, the immunogenicity of the vaccine may be underestimated (26–28). The reason that this is noted more strongly in older children is that within the same pretiter of ≥1:40 category, older children had even higher pretiters.

In previous research, the immune response to IIV3, along with predictive factors such as the pretiter and age, was evaluated by multivariate analysis. Walter et al. used a multivariate logistic model adjusted for multiple factors and reported that in young children aged between 6 and 23 months, increasing age is a significant predictive factor of sP (20). Unfortunately, however, no adjustment using the pretiter was made. In this study, we adjusted for the pretiter and, furthermore, gave older age a significant association with immune response. While the OR of the pretiter adjusted for age differed from the crude OR, the OR of age adjusted for the pretiter gave values extremely close to those of the crude OR. This suggests that the effect of age is certain and that the OR of the pretiter is strongly affected by age. Furthermore, Neuzil et al. performed a multivariate analysis after adjusting for both factors, pretiter and age, and reported that the strongest predictive indicator for immune response after a single vaccination was pretiter and that age was not an independent predictive factor (21). The age range of subjects in their study, however, was between 5 and 8 years, which is older than the ages of the subjects in the

present study. Comparing these results with the results of this study leads us to consider that even in young children less than 8 years of age, immune function develops up to the age of 4 to 5 years and that the effect of age on the immune response in less significant.

The prevaccination GMT for the H3 strain was far higher than those for the other strains, and the postvaccination GMT was also high as a result. This finding is believed to be related to the fact that the H3 strain was the predominant circulating strain during the past four seasons. These findings agree with the results of other studies conducted during the four seasons (17, 18). However, children with a pretiter of <1:10 accounted for 55% even in 2- to 3-year-olds who had a chance of prevaccination exposure, and these children may represent the subpopulation in which the titer does not increase well after exposure. In fact, sR and sP after the first and second vaccine administrations were lower in 2- to 3-year-old children with a pretiter of <1:10 against the H3 strain compared to those with a pretiter of <1:10 against the H1 or B strain (Fig. 2 and 3).

Some limitations associated with this study include the fact that the vaccination dose used was smaller than that regulated for the United States and Europe, along with the fact that the dose used for children between 6 months and 1 year was different from that used for children more than 1-year-old (0.1 ml and 0.2 ml, respectively). It is possible that the seronegative children (pretiter of <1:10) may have had a lower immune response even after the second vaccination as a result of this. Additionally, a simple comparison of the immune responses of children 6 months to 1 years of age with those of older children cannot be made. Despite this, however, within the seronegative group there was a clear difference in the responses between 1-year-olds and those aged 2 to 3 years old, who received the same dose of vaccine, confirming the effect of age. The group with a pretiter of ≥1:10 had almost no children aged between 6 months and 1 year within its distribution, while increases in antibodies in children aged 1 year and older similar to those noted in other studies were seen, thus leading us to believe that the impact of the low dosage is small. Second, with regard to covariate adjustment, given their correlation to age and the sample size, influenza vaccination history and ILI history were not included in the final multivariate analysis in order to construct a stable model. In fact, an adjusted analysis was carried out using both factors; however, since the overall trends remained consistent, we deemed that there was no significant impact on the results.

The viruses causing influenza epidemics differ, depending on the time and place, and as such, the vaccine strain also changes from season to season. In the same manner, the proportion of people possessing antibodies varies, depending on the time, place, and population within which the virus occurs. As a result, just as it is difficult to evaluate effectiveness, it is similarly difficult to evaluate efficacy with immune markers such as antibody titers as a substitute endpoint. Research on adult subjects by Hobson et al. (29) indicates that an HI antibody titer of 1:40 is determined to confer 50% protection against infection (protective level). However, among young children, an HI titer of 1:110 is suggested to be the threshold value for achieving 50% prevention, leading to other reports questioning whether or not a 50% protective effect is in fact sufficient in terms of public health policy (30). An immunological correlation is considered to have been established between the HI antibody titer and the protective effect of influenza; however, this threshold cannot be said to have been firmly established as of yet, particularly with regard to young children.

In summary, we demonstrated in the present study that the pretiter and age are mutually independent predictive factors of the immune response to IIV3 in young children less than 4 years of age. The immunogenicity of the vaccine was low in the young children without prevaccination antibodies and in young children generally. We therefore hope that future studies will evaluate the immune response of vaccine-naive young children to various types of vaccines, including high-dose vaccines other than the split virus type and adjuvant-added vaccines, to improve the immunogenicity of vaccines in this age group. Furthermore, in order to correctly evaluate the immunogenic potential of influenza vaccine in young children, not only the stratification of the HI value 1:40 but also a more detailed stratification, along with stratification according to age are considered important. Additionally, further considerations are required with regard to the HI antibody titer thresholds for immune correlates of vaccine-induced protection in children.

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We declare no conflicts of interest.

REFERENCES

1. **Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, Fukuda K.** 2004. Influenza-associated hospitalizations in the United States. JAMA **292:**1333–1340. http://dx.doi.org/10.1001/jama.292.11.1333.
2. **Izurieta HS, Thompson WW, Kramarz P, Shay DK, Davis RL, DeStefano F, Black S, Shinefield H, Fukuda K.** 2000. Influenza and the rated of hospitalization for respiratory disease among infants and young children. N. Engl. J. Med. **342:**232–239. http://dx.doi.org/10.1056/NEJM200001273420402.
3. **Centers for Disease Control and Prevention (CDC).** 2013. Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices—United States, 2013–2014. MMWR Recomm. Rep. **62**(RR-07):1–43.
4. **Ritzwoller DP, Bridges CB, Shetterly S, Yamasaki K, Kolczak M, France EK.** 2005. Effectiveness of the-2004 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. Pediatrics. **116:**153–159. http://dx.doi.org/10.1542/peds.2005-0049.
5. **Shuler CM, Iwamoto M, Bridges CB, Marin M, Neeman R, Garqiullo P, Keyserling HL, Terebuh PD.** 2007. Vaccine effectiveness against medically attended, laboratory-confirmed influenza among children aged 6 to 59 months, 2003-2004. Pediatrics. **119:**e587–e595. http://dx.doi.org/10.1542/peds.2006-1878.
6. **Allison MA, Daley MF, Crane LA, Barrow J, Beaty BL, Allred N, Berman S, Kempe A.** 2006. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003-2004 season. J. Pediatr. **149:**755–762. http://dx.doi.org/10.1016/j.jpeds.2006.06.036.
7. **Skowronski DM, Hottes TS, Chong M, De Serres G, Scheifele DW, Ward BJ, Halperin SA, Janjua NZ, Chan T, Sabaiduc S, Petric M.** 2011. Randomized controlled trial of dose response to influenza vaccine in children aged 6 to 23 months. Pediatrics. **128:**e276–e289. http://dx.doi.org/10.1542/peds.2010-2777.
8. **Englund JA, Walter EB, Gbadebo A, Monto AS, Zhu Y, Neuzil KM.** 2006. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. Pediatrics. **118:**e579–e585. http://dx.doi.org/10.1542/peds.2006-0201.

9. **Englund JA, Walter EB, Fairchok MP, Monto AS, Neuzil KM.** 2005. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. Pediatrics. **115:**1039–1047. http://dx.doi.org/10.1542/peds.2004-2373.
10. **Walter EB, Neuzil KM, Zhu Y, Fairchok MP, Gagliano ME, Monto AS, Englund JA.** 2006. Influenza vaccine immunogenicity in 6- to 23-month-old children: are identical antigens necessary for priming? Pediatrics. **118:**e570–e578. http://dx.doi.org/10.1542/peds.2006-0198.
11. **Donatelli I, Zannolli R, Fuiano L, Biasio LR.** 1998. Influenza vaccine in immunogenically naive healthy infants. Eur. J. Pediatr. **157:**949–950. http://dx.doi.org/10.1007/s004310050974.
12. **Mitchell DK, Ruben FL, Gravenstein S.** 2005. Immunogenicity and safety of inactivated influenza virus vaccine in young children in-2004. Pediatr. Infect. Dis. J. **24:**925–927. http://dx.doi.org/10.1097/01.inf.0000180978.66362.d9.
13. **Della Cioppa G, Vesilari T, Sokal E, Lindert K, Nicolay U.** 2011. Trivalent and quadrivalent MF59-adjuvanted influenza vaccine in young children: a dose- and schedule-finding study. Vaccine **29:**8696–8704. http://dx.doi.org/10.1016/j.vaccine.2011.08.111.
14. **King JC, Jr., Cox MM, Reisinger K, Hedrick J, Graham I, Patriarca P.** 2009. Evaluation of the safety, reactogenicity and immunogenicity of FluBlok trivalent recombinant baculovirus-expressed hemagglutinin influenza vaccine administered intramuscularly to healthy children aged 6–59 months. Vaccine **27:**6589–6594. http://dx.doi.org/10.1016/j.vaccine.2009.08.032.
15. **Gonzalez M, Pirez MC, Ward E, Dibarboure H, García A, Picolet H.** 2000. Safety and immunogenicity of a paediatric presentation of an influenza vaccine. Arch. Dis. Child. **83:**488–491. http://dx.doi.org/10.1136/adc.83.6.488.
16. **Neuzil KM, Dupont WD, Wright PF, Edwards KM.** 2001. Efficacy of inactivated and cold-adapted vaccines against influenza A infection, to 1985-1990: the pediatric experience. Pediatr. Infect. Dis. J. **20:**733–740. http://dx.doi.org/10.1097/00006454-200108000-00004.
17. **Nolan T, Richmond PC, McVernon J, Skeljo MV, Hartel GF, Bennet J, Basser RL.** 2009. Safety and immunogenicity of an inactivated thimerosal-free influenza vaccine in infants and children. Influenza Other Respir Viruses. **3:**315–325. http://dx.doi.org/10.1111/j.1750-2659.2009.00108.x.
18. **Schmidt-Ott R, Schwarz T, Haase R, Sander H, Walther U, Fourneau M, Htun-Myint L, Sänger R, Schuster V.** 2007. Immunogenicity and reactogenicity of a trivalent influenza split vaccine in previously unvaccinated children aged 6–9 and 10–13 years. Vaccine **26:**32–40. http://dx.doi.org/10.1016/j.vaccine.2007.10.049.
19. **Kumagai T, Nagai K, Okui T, Tsutsumi H, Nagata N, Yano S, Nakayama T, Okuno Y, Kamiya H.** 2004. Poor immune responses to influenza vaccination in infants. Vaccine **22:**3404–3410. http://dx.doi.org/10.1016/j.vaccine.2004.02.030.
20. **Walter EB, Rajagopal S, Zhu Y, Neuzil KM, Fairchok MP, Englund JA.** 2010. Trivalent inactivated influenza vaccine (TIV) immunogenicity in children 6 through 23 months of age: do children of all ages respond equally? Vaccine **28:**4376–4383. http://dx.doi.org/10.1016/j.vaccine.2010.04.058.
21. **Neuzil KM, Jackson LA, Nelson J, Klimov A, Cox N, Bridges CB, Dunn J, DeStefano F, Shay D.** 2006. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5–8-year-old children. J. Infect. Dis. **194:**1032–1039. http://dx.doi.org/10.1086/507309.
22. **Irie S, Fujieda M, Ito K, Ishibashi M, Takamizawa T, Ishikawa T, Takasaki Y, Shindo S, Yokoyama T, Yamashita Y, Shibao K, Koyanagi H, Maeda A, Hirota Y.** 2007. Immunogenicity of trivalent-inactivated influenza vaccine among children less than 4 years old. Kansenshogaku Zasshi. **81:**284–290. (In Japanese.)
23. **World Health Organization.** 2002. WHO manual on animal influenza diagnosis and surveillance. World Health Organization, Geneva, Switzerland. http://www.who.int/csr/resources/publications/influenza/en/whocdscsrncs20025rev.pdf.
24. **European Committee for Proprietary Medicinal Products.** 1997. Note for guidance on harmonisation of requirements for influenza vaccines (CPMP/BWP/214/96). European Agency for the Evaluation of Medicinal Products, London, United Kingdom. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf.
25. **U.S. Department of Health and Human Services, Food and Drug Administration Center for Biologics Evaluation and Research.** 2007. Guidance for industry: clinical data needed to support the licensure of seasonal inactivated influenza vaccines. Office of Communication, Training and Manufacturers Assistance, Rockville, MD. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091985.pdf.
26. **Hirota Y, Kaji M, Ide S, Goto S, Oka T.** 1996. The hemagglutination inhibition antibody responses to an inactivated influenza vaccine among healthy adults: with special reference to the prevaccination antibody and its interaction with age. Vaccine **14:**1597–1602. http://dx.doi.org/10.1016/S0264-410X(96)00153-3.
27. **Chiu SS, Peiris JS, Chan KH, Wong WH, Lau YL.** 2007. Immunogenicity and safety of intradermal influenza immunization at a reduced dose in healthy children. Pediatrics. **119:**1076–1082. http://dx.doi.org/10.1542/peds.2006-3176.
28. **Seidman JC, Richard SA, Viboud C, Miller MA.** 2012. Quantitative review of antibody response to inactivated seasonal influenza vaccines. Influenza Other Respir Viruses. **6:**52–62. http://dx.doi.org/10.1111/j.1750-2659.2011.00268.x.
29. **Hobson D, Curry RL, Beare AS, Ward-Gardner A.** 1972. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J. Hyg. (Lond.) **70:**767–777. http://dx.doi.org/10.1017/S0022172400022610.
30. **Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, Tsai T, Clemens R, Rappuoli R.** 2011. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. Pediatr. Infect. Dis. J. **30:**1081–1085. http://dx.doi.org/10.1097/INF.0b013e3182367662.

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CIRRHOSIS AND LIVER FAILURE

Influenza A(H1N1)pdm09 vaccine effectiveness and other characteristics associated with hospitalization in chronic liver disease patients

Satoko Ohfuji[1], Wakaba Fukushima[1], Yachiyo Sasaki[1], Akihiro Tamori[2], Osamu Kurai[3], Kiyohide Kioka[4], Kazuhiro Maeda[5], Akiko Maeda[1] and Yoshio Hirota[1]

1 Department of Public Health, Osaka City University Faculty of Medicine, Osaka, Japan
2 Department of Hepatology, Osaka City University Faculty of Medicine, Osaka, Japan
3 Department of Gastroenterology and Hepatology, Osaka City Juso Hospital, Osaka, Japan
4 Department of Hepatology, Osaka City General Hospital, Osaka, Japan
5 The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan

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Correspondence
Satoko Ohfuji, MD, PhD, Department of Public Health, Osaka City University Faculty of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan
Tel: +81 6 6645 3756
Fax: +81 6 6645 3757
e-mail: satop@med.osaka-cu.ac.jp

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Abstract

Background & Aims: To date, few studies have investigated the clinical effectiveness of influenza vaccine in chronic liver disease patients. The aim of this study was to examine the effectiveness of monovalent inactivated influenza A (H1N1)pdm09 vaccine and other characteristics associated with hospitalization in patients with chronic hepatitis C. *Methods:* We conducted a hospital-based cohort study during influenza A(H1N1)pdm09 pandemic. A total of 408 patients (132 vaccinated, 276 unvaccinated) with detectable HCV-RNA were followed up with respect to any hospitalization using a weekly postal questionnaire. Reported hospitalizations were verified by medical records. *Results:* During the epidemic period, 28 hospitalizations (6 vaccinated, 22 unvaccinated) were observed. After adjustment for potential confounders, vaccination decreased the odds ratio (OR) for hospitalization with marginal significance (OR = 0.43, 95%CI = 0.16–1.17). Besides, positive association with hospitalization was observed in patients with albumin levels <3.5 g/dl (OR = 8.40, 3.66–19.3) and steroid users (OR = 5.58, 0.98–31.7). *Conclusions:* Among patients with chronic hepatitis C, A(H1N1)pdm09 vaccine appeared to have a protective effect against hospitalization. Those patients with a higher risk for hospitalization should be carefully followed during the influenza season, even when vaccinated.

Patients with chronic liver disease are classified as a high-risk group for influenza-related complications (1, 2). Influenza infection can cause hepatic decompensation and hospitalization in patients with advanced liver disease (3, 4). Thus, preventing severe influenza that requires hospitalization has been an important issue in patients with chronic liver disease.

As influenza vaccination is the most effective method for preventing influenza and its complications, the Advisory Committee on Immunization Practices in the USA has recommended annual influenza vaccination for patients with chronic liver disease since 2007 (5). In Japan, however, no recommendations about influenza vaccination for these patients had been proposed prior to the 2009 influenza A (H1N1) pandemic. One of the reasons for this lack of recommendations might have been little scientific evidence regarding the clinical effectiveness of influenza vaccine among patients with chronic liver disease. To the best of our knowledge, there has been only one study on this topic until now. The Korean study indicated that seasonal influenza vaccine decreased the incidence of laboratory-confirmed influenza and associated symptoms in cirrhotic patients (6). However, no studies so far have demonstrated the effectiveness of influenza vaccine to prevent hospitalization in these patients.

Thus, the primary objective of this study was to examine the effectiveness of influenza A(H1N1)pdm09 vaccine in preventing hospitalization among patients with chronic liver disease. Using these data, the other characteristics associated with hospitalization were also assessed as a secondary objective.

Material and methods

Study subjects

In Japan, monovalent inactivated unadjuvanted split-virus influenza A(H1N1)pdm09 vaccine became available for tiered use in October 16, 2009. Vaccination was scheduled first for healthcare workers, pregnant women and then provided to patients with underlying illnesses (including the present study subjects) from November 2009, according to the order of priority of the groups. The present hospital-based cohort study was performed under the constraint of this national vaccination strategy.

Between November 2009 and January 2010 (i.e. recruitment), patients with chronic hepatitis C who had been under clinical follow-up at three medical institutions in Osaka, Japan, were invited to participate in this study. Eligible patients were those with detectable HCV-RNA levels at the time of recruitment, whereas those with a prior episode of influenza A(H1N1)pdm09 virus infection were excluded. A total of 416 subjects who agreed to participate were enrolled. All study subjects provided their written, informed consent after the nature and possible consequences of this study had been explained.

The study protocol was approved by the Ethics Committees at the Osaka City University Faculty of Medicine, Osaka City Juso Hospital and Osaka City General Hospital, and was performed in accordance with the Declaration of Helsinki.

Information collection

Three kinds of data were collected for each subject. Two kinds of data, physical and environmental characteristics, as well as clinical characteristics, were collected for use as baseline data, whereas data regarding subsequent hospitalization were collected weekly in the follow-up survey. Information on the following physical and environmental characteristics was collected using a self-administered questionnaire: status of influenza A (H1N1)pdm09 vaccination and date of vaccination (if vaccinated); sex, age (years), height (cm) and weight (kg); steroid treatment for two or more consecutive weeks within the last 6 months; underlying illnesses other than liver disease (hereinafter referred to as 'other chronic diseases') including diabetes mellitus, chronic heart disease, chronic renal disease, neuromuscular disease, asthma and chronic respiratory disease; smoking and alcohol habits; number of family members; and total room space in the patient's house (m^2).

In addition, information about clinical characteristics was collected using a structured questionnaire that was completed by the physician-in-charge at the time of recruitment. The questionnaire gathered information about: current treatment with interferon; hepatocellular carcinoma; ascites; hepatic encephalopathy; and laboratory data such as platelet count ($\times 10^4/mm^3$), albumin (g/dl) and prothrombin activity (%). Using these data, Child–Pugh Scores were calculated according to the conventional method (7). Child–Pugh Scores of 5 or more were considered to indicate cirrhosis.

With respect to the follow-up survey, the subjects were requested to fill out a weekly postal questionnaire about the following episodes during the preceding week: physician-diagnosed influenza, results of rapid antigen testing, if applicable, and hospitalization. The postal questionnaire was to be returned to the Department of Public Health, Osaka City University Faculty of Medicine each week during the follow-up period, which was between recruitment and the 15th week of 2010 (April 12–18). For subjects who had been vaccinated within 2 weeks before recruitment, to consider the time length required for a sufficient immune response, the follow-up started 2 weeks after vaccination (8). Reported hospitalizations were verified by medical records at three participating hospitals.

Outcome definitions and epidemic

The study outcome was defined as hospitalization that occurred during the epidemic period of influenza A (H1N1)pdm09. The epidemic period was determined using the surveillance data in Osaka Prefecture and was defined as the period in which the weekly number of influenza patients remained at ≥1 per sentinel (9). Based on the epidemic curve (Fig. 1), the epidemic peaked in November (when this study started) and continued to the 7th week of 2010 (February 15–21). All influenza viruses isolated in Osaka Prefecture during this period were influenza A(H1N1)pdm09 virus strains.

Statistical analysis

Baseline characteristics were compared between vaccinated and unvaccinated subjects using the χ^2 test and the Wilcoxon rank sum test. To evaluate the association between baseline characteristics and outcome, univariate and multivariate logistic regression models were employed to obtain crude and adjusted odds ratios (OR) and their 95% confidence intervals (CI).

In constructing a multivariate model, nine variables were selected for inclusion in the initial model, as three variables were distributed differently between vaccinated and unvaccinated subjects ($P < 0.1$) and the remaining variables were considered medically significant in relation to outcomes. Then, the reduced model was constructed, as the initial model included too many variables for the number of outcome events. In this process, variables that had no association with hospitalization in the results of initial models were excluded. Eventually, the final model included the following four variables: vaccination; other chronic diseases; steroid treatment within the last 6 months; and albumin level.

The results were also verified in the subgroup who was not receiving interferon therapy, as subjects

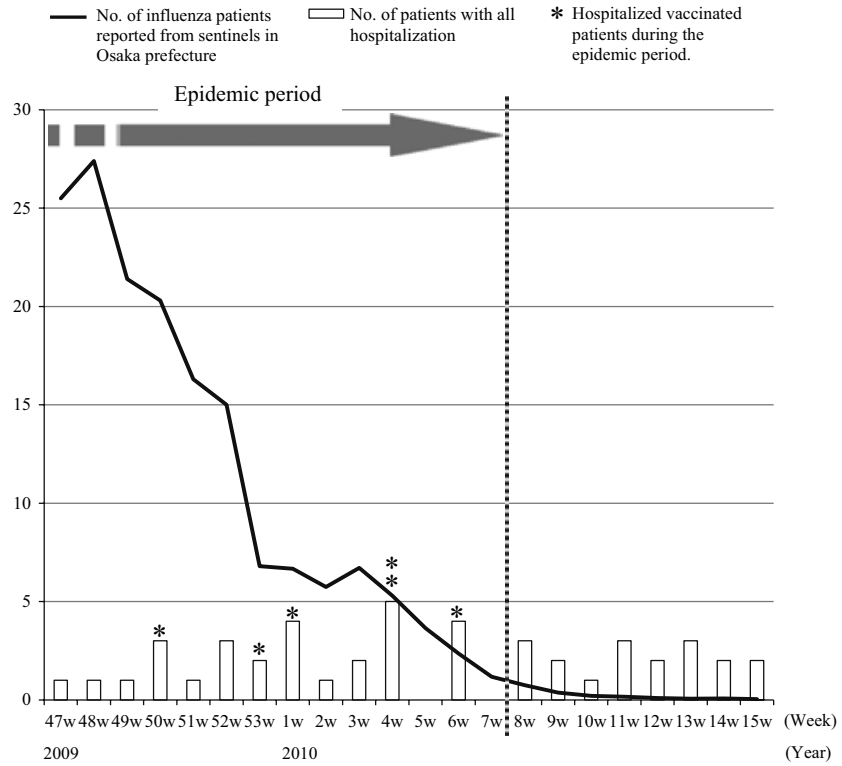

Fig. 1. Data from regional surveillance (line) and from follow-up surveys of study subjects (bars).

receiving interferon therapy were likely to develop influenza-like symptoms because of the side effects of interferon, which might affect the results.

Furthermore, to consider the vaccine effectiveness according to liver function, stratified analysis by platelet counts or albumin levels was also conducted. All tests were two-sided. All analyses were performed using SAS version 9.1.3 software (SAS Institute, Cary, NC, USA).

Results

Baseline characteristics of study subjects

Of the 416 patients with chronic hepatitis C, eight unvaccinated patients (2%) were excluded because of incomplete data in the follow-up surveys. Eventually, data from a total of 408 patients (132 vaccinated, 276 unvaccinated) were analysed.

Table 1 compares baseline characteristics between vaccinated and unvaccinated patients. The vaccinees included a smaller proportion of males (23% vs. 41%, $P < 0.001$), and had less habit of smoking (never smokers: 78% vs. 64%, $P = 0.015$) and alcohol drinking (never-drinkers: 77% vs. 66%, $P = 0.038$). Variables that were thought to be potentially associated with influenza, such as age, body mass index, steroid treatment, other chronic diseases, room space per person, interferon treatment, hepatocellular carcinoma and laboratory data suggesting cirrhosis, were distributed similarly between the vaccinated and unvaccinated patients.

Association of influenza A(H1N1)pdm09 vaccine with hospitalization

Figure 1 shows the distribution of outcome events from the follow-up surveys of study subjects (bars). During the epidemic period (from the 47th week of 2009 to the 7th week of 2010), there were 28 hospitalizations (7%), including 6 vaccinated patients.

Table 2 shows the crude and adjusted ORs of influenza vaccine for hospitalization during the epidemic period. Compared with unvaccinated patients, vaccinated lowered the OR for hospitalization to about half in the crude analysis (OR = 0.55, 95%CI = 0.22–1.39). After adjustment for potential confounders, the decreased OR of vaccination reached the marginally significant level (OR = 0.43, 95%CI = 0.16–1.17). Even in the subjects who were not receiving interferon therapy, both the proportion of outcome events and the ORs of vaccination were almost the same as for the entire study subjects. However, ORs of vaccination somewhat fluctuated according to their liver function, and subjects with better liver disease status (i.e. platelet count $\geq 10.0 \times 10^4/mm^3$ or albumin level ≥3.5 g/dl) seemed to be more likely to manifest vaccine effectiveness. Especially in subjects with platelet count $\geq 10.0 \times 10^4/mm^3$, vaccination was associated with a decreased OR for

Table 1. Comparison of baseline characteristics between patients with influenza A(H1N1)pdm09 vaccination and those without vaccination

Characteristics	Category	Vaccinated (n = 132)	Unvaccinated (n = 276)	P *
Sex	Male	31 (23)	112 (41)	<0.01
Age (years)	65.0+	85 (64)	176 (64)	0.90
Body mass index (kg/m^2)	25.0+	20 (15)	49 (18)	0.49
	Data missing		2	
Steroid treatment within the last 6 months	Received	5 (4)	5 (2)	0.22
	Data missing	1		
Underlying illness other than liver disease		57 (43)	106 (39)	0.37
Diabetes mellitus		14 (11)	33 (12)	0.68
Chronic heart disease		11 (8)	15 (5)	0.27
Chronic renal disease		7 (5)	13 (5)	0.80
Neuromuscular disease		7 (5)	10 (4)	0.43
Malignant neoplasm		3 (2)	14 (5)	0.29
Asthma		6 (5)	8 (3)	0.40
Blood dyscrasia		3 (2)	11 (4)	0.37
Others†		8 (6)	20 (7)	0.65
	Data missing		1	
Smoking habit	Never	103 (78)	176 (64)	0.02
	Ever	16 (12)	53 (19)	
	Current	13 (10)	47 (17)	
Alcohol drinking habit	Never	102 (77)	181 (66)	0.04
	Ever	21 (16)	57 (21)	
	Current	9 (7)	38 (14)	
Room space per person (m^2)	Mean (SD)	43.2 (25.9)	40.8 (26.8)	0.37
	Unknown	3	6	
Clinical characteristics at the time of recruitment				
Interferon treatment	Receiving	40 (30)	105 (38)	0.12
	Data missing		1	
Hepatocellular carcinoma	Present	26 (20)	56 (21)	0.82
	Data missing		5	
Laboratory data				
Platelet count ($\times 10^4$/mm^3)	<10.0	42 (32)	89 (32)	0.89
	Data missing		2	
Albumin level (g/dl)	<3.5	22 (17)	41 (15)	0.61
	Data missing	1		
Prothrombin activity (%)	<80	19 (18)	23 (13)	0.35
	Data missing	24	105	
Child–Pugh score	5+	43 (40)	72 (42)	0.68
	A (5–6)	37 (34)	62 (36)	0.80
	B (7–9)	5 (5)	10 (6)	
	C (10+)	1 (1)	0 (0)	
	Data missing	24	106	

SD, standard deviation. Data expressed as n (%) unless otherwise indicated.

*The χ^2 test or Wilcoxon rank sum test was employed where appropriate.

†Others included 11 atopic disease, 7 pregnancy, 5 collagen disease, 4 cerebrovascular disease, 3 chronic respiratory disease and 1 immunosuppressive disease.

hospitalization with marginal significance (OR = 0.19, 95%CI = 0.03–1.22).

Association of other clinical variables with hospitalization

Table 3 shows the association of other baseline characteristics with hospitalization during the epidemic period. Patients with other chronic diseases had about a two-fold increased OR for hospitalization with marginal significance in the crude analysis (crude OR = 2.10, 95%CI = 0.97–4.57). After adjustment for potential confounders, however, the increased OR was not significant. Instead, OR of steroid use showed a marginal association with hospitalization (adjusted OR = 5.58, 95% CI = 0.98–31.7). In addition, patients with a lower albumin level had significantly increased ORs for hospitalization both in the crude and adjusted analyses (adjusted OR = 8.40, 95%CI = 3.66–19.3).

Other liver function markers were also investigated by incorporating them into the model instead of the albumin level, as the positive association between a lower albumin level and hospitalization seemed to represent an

Table 2. Association of influenza A(H1N1)pdm09 vaccine with hospitalization during the epidemic period, according to the selected clinical condition subgroup: crude and adjusted analyses

Stratified category	Vaccination status	*N*	*n* (%)	Crude OR (95%CI)	Adjusted* OR (95%CI)
Entire study subjects	Unvaccinated	276	22 (8)	1.00	1.00
	Vaccinated	132	6 (5)	0.55 (0.22–1.39)	0.43 (0.16–1.17)
Interferon therapy					
Not receiving	Unvaccinated	170	15 (9)	1.00	1.00
	Vaccinated	92	5 (5)	0.59 (0.21–1.69)	0.43 (0.14–1.35)
Receiving	Unvaccinated	105	7 (7)	1.00	1.00
	Vaccinated	40	1 (3)	0.36 (0.04–3.01)	0.40 (0.04–3.87)
Platelet count ($\times 10^4/mm^3$)					
≥ 10.0	Unvaccinated	185	12 (6)	1.00	1.00
	Vaccinated	90	2 (2)	0.33 (0.07–1.50)	0.19 (0.03–1.22)
<10.0	Unvaccinated	89	10 (11)	1.00	1.00
	Vaccinated	42	4 (10)	0.83 (0.25–2.82)	0.75 (0.21–2.66)
Albumin level (g/dl)					
≥3.5	Unvaccinated	235	13 (6)	NA	NA
	Vaccinated	109	0 (0)		
<3.5	Unvaccinated	41	9 (22)	1.00	1.00
	Vaccinated	22	6 (27)	1.33 (0.40–4.40)	1.13 (0.32–4.00)

OR, odds ratio; CI, confidence interval; NA, not applicable.
*Model includes underlying illnesses other than liver disease, steroid treatment within the last 6 months and albumin level, other than the stratified variable.

Table 3. Association of influenza A(H1N1)pdm09 vaccine and other baseline characteristics with hospitalization during the epidemic period: crude and adjusted analyses

Baseline characteristics	*n* (%)	Crude OR (95%CI)	Adjusted* OR (95%CI)
Vaccination status			
Unvaccinated	22 (8)	1.00	1.00
Vaccinated	6 (5)	0.55 (0.22–1.39)	0.43 (0.16–1.17)
Underlying illness other than liver disease			
Absent	12 (5)	1.00	1.00
Present	16 (10)	2.10 (0.97–4.57)	1.82 (0.80–4.14)
Steroid treatment within the last 6 months			
Not received	26 (7)	1.00	1.00
Received	2 (20)	3.57 (0.72–17.7)	5.58 (0.98–31.7)
Albumin level (g/dl)			
<3.5	15 (24)	7.96 (3.57–17.7)	8.40 (3.66–19.3)
3.5+	13 (4)	1.00	1.00

OR, odds ratio; CI, confidence interval; NA, not applicable.
*Model includes all variables in this table.

association with advanced liver disease. The adjusted ORs for hospitalization of any liver function markers were also increased: platelet count $<10.0 \times 10^4/mm^3$ (OR = 2.10, 95%CI = 0.96–4.60), prothrombin activity <80% (OR = 4.32, 95%CI = 1.69–11.1), Child–Pugh Score of 5 or more (OR = 3.51, 95%CI = 1.38–8.92) and hepatocellular carcinoma (OR = 3.09, 95%CI = 1.38–6.91).

Discussion

In this study among patients with chronic hepatitis C, there was an indication of vaccine effectiveness for preventing severe outcomes requiring hospitalization during an epidemic. Although the limited number of outcome events made it difficult to detect significant vaccine effectiveness, the present results support the usefulness of influenza vaccine for patients with chronic hepatitis C.

To date, no study has reported the effectiveness of influenza A(H1N1)pdm09 vaccine against hospitalization in patients with specific underlying medical conditions including chronic liver disease. However, based on the reports about vaccine effectiveness among subjects with any high-risk condition, a cohort study in Denmark showed that vaccine conferred protection against influenza-related hospitalization to 44% (−19–73%) among subjects <65 years with underlying illnesses (10). A matched case–control study in the Netherlands indicated that the vaccine effectiveness for influenza-related hospitalization was 19% (−28–49%) among subjects with

high-risk conditions (11). Although these studies did not refer to vaccine effectiveness in patients with individual underlying illnesses, the present results among patients with chronic hepatitis C would correspond to those in subjects with any high-risk conditions.

Influenza infection occasionally causes hepatic decompensation without typical influenza symptoms in patients with chronic liver disease (4, 6), which might bring about delayed antiviral therapy and increase influenza-related mortality. Thus, it was an important finding that influenza vaccine had some effect for reducing hospitalization during the epidemic period, although the present results were not significant. According to the previous studies, vaccination for cirrhotic patients lowered the incidence of laboratory-confirmed influenza and atypical influenza symptoms such as myalgia, hepatic decompensation, oliguria and uncontrolled ascites during influenza season (6). Furthermore, some reports have indicated that influenza vaccine was sufficiently immunogenic in patients with cirrhosis (12–15). Taken together, it would be reasonable to advise vaccination for patients with chronic liver disease. In fact, the Advisory Committee on Immunization Practices in the USA has recommended annual influenza vaccination for patients with chronic liver disease since 2007 (5), and the WHO position paper has indicated that patients with specific chronic medical conditions continue to be an appropriate target group for annual influenza vaccination (16).

In this study, however, subjects with advanced liver disease (represented by lower albumin level) had a higher risk for hospitalization during the epidemic period, irrespective of their vaccination status (Table 3). These results corresponded to a previous case report in which influenza infection caused hepatic decompensation and hospitalization in patients with advanced liver disease (4). Influenza virus itself could cause hepatitis (17), and influenza infection could induce toxic metabolites and proinflammatory cytokines such as TNF-α, IL-1 and IL-6, which contribute to hepatic damage (18, 19). These seemed to result in disease deterioration, especially in patients with advanced liver disease. Thus, it would be better for subjects with advanced liver disease to be followed with special attention during the season, even when vaccinated.

In addition, steroid treatment and the presence of other chronic diseases were related to hospitalization during the epidemic period, independent of vaccination status or liver function. Steroid treatment and the presence of chronic diseases have been the known high-risk factors for influenza and its complications (5). In the 2009 influenza pandemic, immunosuppressive therapy and chronic diseases (especially asthma) were among the highest comorbid conditions in critically ill patients with influenza A(H1N1)pdm09 infection in the USA (20), Canada (21), Australia (22) and Mexico (23). The present results agreed with these findings. Patients on immunosuppressive therapy have impaired vaccine responses (24), and patients with asthma are expected to have similar vaccine responses, as they often receive steroid treatment. These backgrounds of poor immunological responses might bring about the high sensitivity for influenza infection and severe outcomes owing to influenza.

When interpreting the present results, however, the following limitations should be considered. Firstly, the insufficient statistical power owing to the small sample size and the limited number of outcome events is obviously important. This limitation made it difficult to detect significant vaccine effectiveness. If more subjects could be recruited, more meaningful results would be obtained. However, studies on pandemic influenza vaccine must be conducted under strict time constraints, as pandemic influenza virus had circulated and pandemic influenza vaccines became available during the epidemic. In addition, the epidemic subsided after sufficient distribution of the vaccines. This tight time schedule represented a major obstacle to recruiting a sufficient number of vaccinated and unvaccinated subjects for any observational prospective cohort study.

Secondly, voluntary enrolment in the observational study might lead to selection bias in the vaccination status. In fact, female patients, non-smokers and non-drinkers tended to receive vaccination in this study, which might lead to a healthy vaccinee effect. However, even when additional analyses that adjusted for these variables were conducted, similar results were obtained (ORs of vaccination were 0.45 (95%CI = 0.16–1.26). On the other hand, the determination of vaccination status relied on patients' self-reports and could not be confirmed by their medical records, as patients usually received any vaccination in their neighbouring clinic. Thus, some non-differential misclassification in the vaccination status might have occurred.

Thirdly, there might be some concern about outcome misclassification, as hospitalization is a less specific outcome for influenza. In this study, however, the methods in which outcomes were confined into the epidemic period would have helped to minimize outcome misclassification and obtain a higher specificity of influenza for hospitalization. Furthermore, hospitalization is essentially considered an objective outcome that can be verified by the medical records, and therefore misclassification owing to non-influenza illness, if any, would be non-differential between vaccinated and unvaccinated patients (25). Such misclassification leads to an underestimation of vaccine effectiveness and does not materially affect the validity of the results.

Finally, previous immunity in unvaccinated patients might affect the underestimation of vaccine effectiveness to some extent. Based on a serological study, about one-third of subjects aged ≥65 years was reported to have pre-existing antibody before the epidemic, as many had been exposed to antigens similar to influenza A(H1N1) pdm09 virus during childhood (26). In this study, however, although about two-thirds of subjects were ≥65 years old, the proportion of subjects with pre-existing

antibody was expected to be lower than in previous studies, because the immunogenicity study of influenza A(H1N1)pdm09 vaccine, in which part of this study subjects participated, indicated that only about 5% of subjects had the pre-existing antibody at the beginning of the pandemic (12). Thus, the effect of previous immunity, if any, would be very minimal.

In conclusion, among patients with chronic hepatitis C, influenza A(H1N1)pdm09 vaccine was suggested to have some protective effect against hospitalization during the epidemic period. As patients with advanced liver disease, steroid treatment and other chronic diseases (especially asthma) are considered to be at higher risk for hospitalization during the epidemic period, they should be followed up with special attention during the season, even when vaccinated.

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Conflicts of interest: The authors do not have any disclosures to report.

References

1. Tomizuka T, Takayama Y, Shobayashi T, Fukushima Y, Suzuki Y. Underlying medical conditions and hospitalization for pandemic (H1N1) 2009, Japan. *Emerg Infect Dis* 2010; **16**: 1646–7.
2. Helferty M, Vachon J, Tarasuk J, *et al.* Incidence of hospital admissions and severe outcomes during the first and second waves of pandemic (H1N1) 2009. *CMAJ* 2010; **182**: 1981–7.
3. Naseem A, Satti S, Khan MA, Saeed W. A clinical account of hospitalized 2009 pandemic influenza A (H1N1) cases. *J Coll Physicians Surg Pak* 2011; **21**: 97–102.
4. Duchini A, Viernes ME, Nyberg LM, Hendry M, Pockros PJ. Hepatic decompensation in patients with cirrhosis during infection with influenza A. *Arch Intern Med* 2000; **160**: 113–5.
5. Fiore AE, Shay DK, Haber P, *et al.* Advisory Committee on Immunization Practices (ACIP), Centers for Disease Control and Prevention (CDC). Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007; **56**: 1–54.
6. Song JY, Cheong HJ, Ha SH, *et al.* Clinical impact of influenza immunization in patients with liver cirrhosis. *J Clin Virol* 2007; **39**: 159–63.
7. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646–9.
8. Bridges CB, Katz JM, Levandowski RA, Cox NJ. Influenza vaccine (inactivated). In: Plotkin S, Orenstein W, Offit P, eds. *Vaccines*. Philadelphia: Elsevier, 2008: 259–90.
9. Osaka Prefectural Institute of Public Health. The weekly number of clinical influenza cases reported by sentinels. Available at: http://www.iph.pref.osaka.jp/infection/2-old.html. Accessed 23 May 2011.
10. Emnorg HD, Krause TG, Hvvid A, Simonsen J, Molbak K. Effectiveness of vaccine against pandemic influenza A/H1N1 among people with underlying chronic diseases: cohort study, Denmark, 2009/10. *BMJ* 2011; **344**: d7901.
11. Steens A, Wijnans EG, Dieleman JP, *et al.* Effectiveness of a MF-59TM-adjuvanted pandemic influenza vaccine to prevent 2009 A/H1N1 influenza-related hospitalization; a matched case-control study. *BMC Infect Dis* 2011; **11**: 196.
12. Ohfuji S, Fukushima W, Tamori A, *et al.* Immunogenicity of influenza A(H1N1)pdm09 vaccine and the associated factors on lowered immune response in patients with hepatitis C. *Influenza Other Respi Viruses* 2012; **7**: 456–65.
13. Gaeta GB, Pariani E, Amendola A, *et al.* Influenza vaccination in patients with cirrhosis and in liver transplant recipients. *Vaccine* 2009; **27**: 3373–5.
14. Gaeta GB, Stornaiuolo G, Precone DF, Amendola A, Zanetti AR. Immunogenicity and safety of an adjuvanted influenza vaccine in patients with decompensated cirrhosis. *Vaccine* 2002; **20**: B33–5.
15. Soesman NMR, Rimmelzwaan GF, Nieuwkoop NL, *et al.* Efficacy of influenza vaccination in adult liver transplant recipients. *J Med Virol* 2000; **61**: 85–93.
16. World Health Organization. Vaccines against influenza WHO position paper-November 2012. *Wkly Epidemiol Rec* 2012; **87**: 461–76.
17. Jain S, Kamimoto L, Bramley AM, *et al.* Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. *N Engl J Med* 2009; **361**: 1935–44.
18. Duchini A, Hendry RM, Redfield DC, Pockros PJ. Influenza infection in patients before and after liver transplantation. *Liver Transpl* 2000; **6**: 531–42.
19. Fukui H. Relation of endotoxin, endotoxin binding proteins and macrophages to severe alcoholic liver injury and multiple organ failure. *Alcohol Clin Exp Res* 2005; **29**: S172–9.
20. Louie JK, Acosta M, Winter K, *et al.* for the California Pandemic (H1N1) Working Group. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. *JAMA* 2009; **302**: 1896–902.
21. Kumar A, Zarychanski R, Pinto R, *et al.* for the Canadian Critical Care Trials Group H1N1 Collaborative. Critically ill patients with 2009 influenza A(H1N1) infection in Canada. *JAMA* 2009; **302**: 1872–9.
22. The ANZIC influenza investigators. Critical care services and 2009 H1N1 influenza in Australia and New Zealand. *N Engl J Med* 2009; **361**: 1925–34.
23. Dominguez-Cherit G, Lapinsky SE, Macias AE, *et al.* Critically ill patients with 2009 influenza A(H1N1) in Mexico. *JAMA* 2009; **302**: 1880–7.
24. Kunisaki KM, Janoff EN. Influenza in immunosuppressed populations: a review of infection frequency, morbidity, mortality, and vaccine responses. *Lancet Infect Dis* 2009; **9**: 493–504.
25. Nichol KL, Margolis KL, Wuorenma J, von Sternberg T. The efficacy and cost effectiveness of vaccination against influenza among elderly persons living in the community. *N Engl J Med* 1994; **331**: 778–84.
26. Hancock K, Veguilla V, Lu X, *et al.* Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. *N Engl J Med* 2009; **361**: 1945–52.

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RESEARCH ARTICLE Open Access

Pertussis outbreak in university students and evaluation of acellular pertussis vaccine effectiveness in Japan

Megumi Hara[1*], Mami Fukuoka[2], Katsuya Tashiro[3], Iwata Ozaki[4], Satoko Ohfuji[5], Kenji Okada[6], Takashi Nakano[7], Wakaba Fukushima[5] and Yoshio Hirota[5,8]

Abstract

Background: Recent studies worldwide have reported increasing numbers of adults diagnosed with *Bordetella pertussis* despite receiving childhood vaccinations. This study describes a pertussis outbreak at a university medical faculty campus and examines the effectiveness of diphtheria, tetanus, and pertussis (DTaP) vaccination completed during infancy in Japan.

Methods: After the outbreak, self-administered questionnaires and serum samples were collected from students on campus to determine the incidence of pertussis and underlying diseases. Pertussis was diagnosed on the basis of clinical criteria and serum anti-pertussis toxin antibody levels. Using data collected from 248 first and second grade students who had submitted copies of their vaccination records, we evaluated the effectiveness of DTaP vaccination in infancy against adult pertussis.

Results: Questionnaire responses were obtained from 636 students (of 671 registered students; 95% response rate). Of 245 students who reported a continuous cough during the outbreak period, 84 (attack rate: 13.2%) were considered "probable" pertussis cases that met clinical criteria. The outbreak occurred mainly in first and second grade students in the Faculty of Medicine. Of 248 students who provided vaccination records, 225 had received 4 DTaP doses (coverage: 90.7%); the relative risk of the complete vaccination series compared to those with fewer than 4 doses or no doses for probable cases was 0.48 (95% confidence interval: 0.24-0.97).

Conclusions: Waning protection was suspected due to over time. Booster vaccination for teenagers and development of highly efficacious pertussis vaccines are needed.

Keywords: Pertussis, Outbreak, Vaccine effectiveness

Background

Although global vaccination coverage for diphtheria, tetanus, and pertussis (DTaP) remains high, recent reports of increasing pertussis cases among adolescents and adults are of concern because this population can be a source of infant infection [1]. Suggested causes for this increase include increased clinical awareness of pertussis, improved diagnostics using polymerase chain reaction (PCR), identification of mutations in the strain of *Bordetella pertussis* associated with epidemics, and decreasing antibody titers after vaccination [2-6]. Western countries have initiated tetanus, reduced-antigen-content diphtheria, and acellular pertussis (Tdap) vaccine booster programs for adolescents, adults, and other high-risk groups [1,7,8].

The number of adult pertussis cases has been increasing in Japan, with outbreaks in high schools and universities as well as workplaces successively reported in 2002 [9-13]. In response to these reports, studies have examined outbreak characteristics, genetic characteristics of *B. pertussis*, and alternative diagnostic methods. However, to our knowledge, no study has evaluated the effectiveness of the current DTaP vaccine. Japan has a different schedule to western countries for baby

* Correspondence: harameg@cc.saga-u.ac.jp
[1]Department of Preventive Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga City, Saga 849-8501, Japan
Full list of author information is available at the end of the article

immunizations, including DTaP vaccine. Until 2012, pertussis vaccination is a triple DTaP vaccine (after 2012, DTaP-IPV), beginning at 3 months of age. To establish initial immunity, 3 times for 3 to 8 weeks apart are needed. A booster dose is given at 6 months to 12 months after the initial immunity. Thus, DTaP vaccine is usually completed by 18 months of age. The recommended number of doses is smaller than that in Western countries. In addition, Tdap booster vaccines are not administered after early adolescence in Japan. To determine the necessity for booster vaccination in early adolescence, it is important to evaluate the effectiveness of the current vaccine program in preventing pertussis after early adolescence. However, there have been a limited number of epidemiological evaluations on vaccine program effectiveness against pertussis in Japan, and these studies have focused primarily on children [14,15]. To our knowledge, no studies have examined the effectiveness of the vaccine against pertussis after early adolescence.

In April 2010, a pertussis outbreak was confirmed among students at the medical faculty campus of Saga University. After the outbreak ended, a retrospective cohort study was performed. This study describes the outbreak and examines the association between infant DTaP vaccination and incidence of pertussis.

Methods

Study populations

More than 20 students visited the health administration center at the Saga University Faculty of Medicine in April 2010 complaining of coughs that had lasted at least 2 weeks. Three of these students had throat swabs positive for *B. pertussis* by loop-mediated isothermal amplification [16]. Thus, this outbreak of cough symptoms was considered to be due to pertussis. The health administration center discouraged club activities, meetings, and ball game tournaments; promoted use of facemasks; terminated practical training for students with coughs; actively encouraged medical examinations at medical institutions; and notified students and faculty members of the outbreak by e-mail. By early July, no new cough cases were reported to the health administration center.

Just after the end of the outbreak in early July, a total of 671 students (411 and 260 from the departments of medicine and nursing, respectively) from the first through fourth grades on the faculty of medicine campus were provided an oral explanation of the purpose, content, and conditions of cooperation of the study, and asked to provide written informed consent forms with agreement to participate. Among them, 636 students (collection rate: 95%) completed a questionnaire about relevant demographic variables and clinical symptoms of cough, including duration, presence of coughing paroxysms, whooping and vomiting after cough, medical institution visits, past history of disease, and DTaP vaccination status. They were also asked to provide serum specimens. Serum samples were obtained from 516 (77.1%) of these students; anti-pertussis toxin (PT) antibody levels were tested by enzyme immunoassay at an outside laboratory (SRL, Inc., Tokyo).

Of these, 248 first and second grade students had submitted copies of their vaccination records, including infant DTaP vaccine administration histories, from their maternity record books to the health administration center upon entering the school. In Japan, vaccination histories are recorded in maternity record books maintained by individuals.

This study design was approved by the ethical review board of the Saga Medical School Faculty of Medicine, Saga University (approval number 22–25, 2010).

Case definitions

Cases were categorized on the basis of 2 clinical definitions of pertussis, using clinical criteria established by the Centers for Disease Control and Prevention and the Council of State and Territorial Epidemiologists 2014 case definitions [17]. "Probable cases" had cough illness lasting ≥2 weeks with at least 1 of the following signs or symptoms: paroxysms of coughing, or inspiratory "whoop", or posttussive vomiting. "Suspected cases" met at least 1 of the 4 clinical symptoms or signs. In addition to these clinical definitions, the serological diagnosis of pertussis required serum anti-PT antibody levels after the outbreak to be higher than 100 EU/mL.

Vaccine effectiveness

The 248 students whose vaccination records could be confirmed by their maternity record books were classified into 2 groups: those who had completed the full 4-dose vaccination as recommended by the Japanese government, those who had received less than 4 vaccine doses or no doses. The attack rate (AR) of pertussis and the relative risk (RR) after 4 doses compared with less than 4 doses or no doses were calculated. The effectiveness of the vaccine was calculated using the equation:

$$(1 - [AR_{vaccinated}/AR_{unvaccinated}]) \times 100\,(\%) = (1 - RR) \times 100\,(\%)$$

Statistical analysis

We used SAS 9.3 for Windows (SAS Institute, Cary, NC, USA) for statistical comparisons between each variable using chi-square and Fisher's exact tests. RRs after 4 doses compared with less than 4 doses or no doses and corresponding 95% confidence intervals (CIs) were obtained using the PROC FREQ procedure in the software

package. RRs and their 95% CIs adjusted by faculty were obtained using the Mantel–Haenszel method.

Results

Description of outbreak

The population characteristics and cough statuses of 636 subjects who participated in the survey just after the outbreak were examined according to clinical diagnosis (Table 1). Among 245 students (38.5%) who presented with a cough during the outbreak period, the most common cough duration was 2 weeks or more, followed by duration of 1–2 weeks. The most common characteristic was paroxysmal cough, followed by posttussive vomiting. On the basis of the reported clinical symptoms, 84 and 161 students were classified into the probable case (mean age 20.4, range: 18–34 years) and suspected case (mean age 20.0, range: 18–30 years) groups, respectively. The number of cases was greatest in first grade students in the Department of Medicine. Of 245 students with continuous cough, 121 visited a medical institution; of these, 56 were diagnosed with pertussis by physicians. Patients with probable cases were more likely to seek treatment at a medical institution and be diagnosed with pertussis than those with suspected cases. Of the students diagnosed with pertussis, 21 had visited the infection control department at the university hospital. Pertussis DNA was detected in throat swabs obtained from 3 of these students by loop-mediated isothermal

Table 1 Characteristics of 636 survey subjects according to clinical diagnosis

Characteristics		Total (n = 636)		No symptoms (n = 391)		Suspected cases (n = 161)		Probable cases (n = 84)		P-value*
		n	(%)	n	(%)	n	(%)	n	(%)	
Department	Medicine	389	61.2	224	57.3	107	66.5	58	69.0	0.037
	Nursing	247	38.8	167	42.7	54	33.5	26	31.0	
Grade	1	164	25.8	85	21.7	54	33.5	25	29.8	0.079
	2	158	24.8	96	24.6	59	36.6	23	27.4	
	3	156	24.5	105	26.9	35	21.7	16	19.0	
	4	158	24.8	105	26.9	33	20.5	20	23.8	
Sex	Male	243	38.2	152	38.9	56	34.8	35	41.7	0.517
	Female	392	61.6	238	60.9	105	65.2	49	58.3	
	Unknown	1	0.2	1	0.3	0	0.0	0	0.0	
Continuous cough	Yes	245	38.5	0		161	100.0	84	100.0	
	Less than 1 week	38	6.0	-		38	23.6	0	0.0	<0.001
	1 week or more and less than 2 weeks	102	16.0	-		102	63.4	0	0.0	
	2 weeks or more	105	16.5	-		21	13.0	84	100.0	
Characters of continuous cough (multiple answers)	Proxysms of coughing	233	36.6	-		152	94.4	81	96.4	0.48
	Inspiratory whooping	22	3.5	-		12	7.5	10	11.9	0.247
	Posttussive vomiting	70	11.0	-		31	19.3	39	46.4	<0.0001
Medical institution	Visited	121	19.0	-		67	41.6	54	64.3	0.0008
	Diagnosed with pertussis	56	8.8	-		30	18.6	26	31.0	0.0323
Self-report DTaP vaccination status										
	No	7	1.1	5	1.3	2	1.2	0	0.0	<0.001
	1 shot	19	3.0	9	2.3	4	2.5	6	7.1	
	2 doses	19	3.0	8	2.0	3	1.9	8	9.5	
	3 doses	10	1.6	5	1.3	5	3.1	0	0.0	
	4 doses	47	7.4	20	5.1	15	9.3	12	14.3	
	Uncertain	534	84.0	344	88.0	132	82.0	58	69.0	

Clinical criteria: (1) cough illness lasting ≧ 2 weeks; (2) paroxysms of coughing; (3) inspiratory "whoop"; (4) post-tussive vomiting.
Suspected case: patient with at least 1 clinical criterion.
Probable case: patient with cough illness lasting ≥ 2 weeks with at least 1 other clinical criterion.
*Chi-square test.

amplification, leading to a definitive laboratory diagnosis of pertussis. Most students (534 of 636) could not remember their vaccination status.

The epidemic curve based on the date of cough onset is shown in Figure 1. The number of individuals with cough symptoms increased rapidly from early April and decreased after peaking from April 19 to 25. No prophylaxis antibiotics were administered during this time.

Figure 2 shows the distribution of anti-PT antibody titers in 516 students from whom serum was collected after the outbreak, according to grade. Among them 24 subjects' anti-PT antibody levels were greater than 100 (EU/mL), and the percentage of them was highest in first grade students.

Evaluation of vaccine effectiveness

Among entire population, 248 first and second grade students whose infant vaccination records could be confirmed by maternity record books were examined according to clinical diagnosis (Table 2). Probable cases were more common in the Department of Medicine. No significant associations were found between grade, sex, and underlying disease and incidence of pertussis. The percentage of students diagnosed with pertussis who had also received the full recommended DTaP vaccination course in infancy was notably low (12.5%). The AR of probable cases per vaccination status was 33% in unvaccinated students and 13.8% in students who had received all 4 doses, indicating that ARs were lowest in students who had received the recommended number of vaccine doses.

There were no statistically significant differences in the department, grade, sex, or underlying diseases compared to the completeness of the infant vaccination series. A significantly higher proportion of individuals who did not receive 4 doses of DTaP reported coughing paroxysms. While the clinical characteristics of cough varied, the proportion of students with anti-PT antibody levels greater than 100 EU/mL after the outbreak were similar between those who did and those who did not receive a full vaccine dose (see Additional file 1: Table S1). We examined the RR of the DTaP vaccine for those who had received the government-recommended number of vaccinations in infancy (Table 3). When outcome was defined as probable cases based on the clinical criteria, the RR for students with 4 doses compared to those with fewer than 4 doses or no doses was 0.48 (95% CI: 0.24–0.97); after adjusting for the impact of department the effectiveness was estimated to be 52% (95% CI: 3–76). Similarly, when outcome was defined as meeting at least 1 of the 4 clinical criteria in both probable and suspected cases, the adjusted RR was 0.70 (95% CI: 0.51–0.98). When outcomes were defined as serological diagnosis of pertussis after the outbreak (anti-PT antibody levels greater than 100 EU/mL) or diagnosed at medical institutions, the RRs were 0.64 (95% CI: 0.16–2.52) and 0.74 (95% CI: 0.21–2.61), respectively; no statistically significant protective effect of complete vaccination were detected using these outcome definitions.

Discussion

The outbreak in this study occurred mainly in first and second grade students on the university campus, with peak incidence in April. Welcoming parties for new pupils or invitations to club activities before and after entrance ceremonies likely contributed to the spread of infection. The outbreak ended without administration of preventive antibiotics. Measures such as self-restraint of club activities, meetings, and ball game tournaments, termination of practical trainings, and active intervention by the health administration center to encourage examination at medical institutions appeared to effectively limit

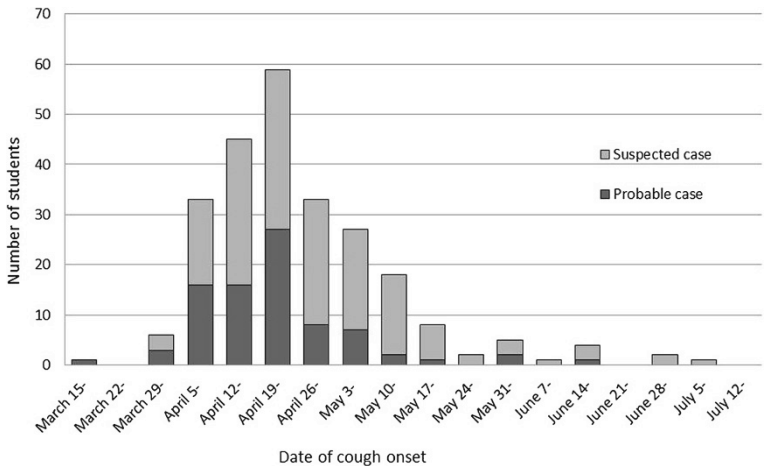

Figure 1 Epidemic curve based on date of cough onset. Dark gray and light gray bars indicate suspected and probable cases, respectively.

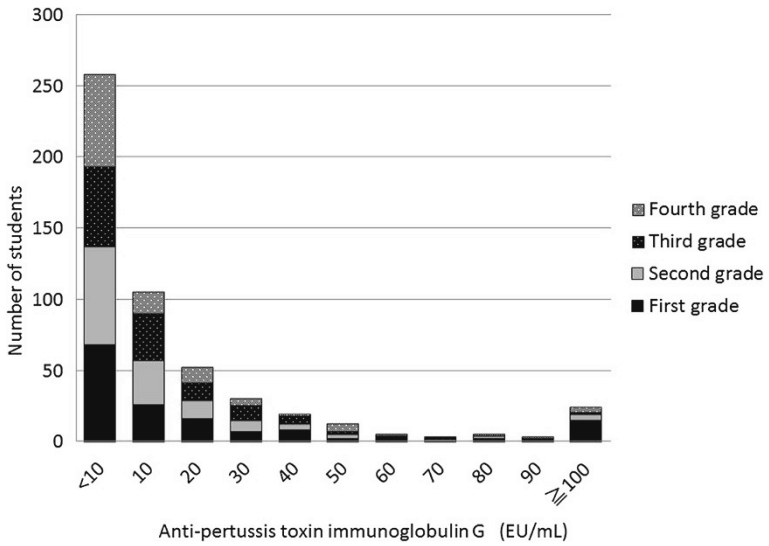

Figure 2 Distribution of serum pertussis toxin antibodies in 516 students after the outbreak, according to grade. Black, gray, black with dots, and gray with dots bars indicate first, second, third, and fourth grades, respectively.

Table 2 Comparison of underlying disease and DTaP vaccination according to clinical diagnosis in 248 students with confirmed vaccination records

Characteristics		Total	No symptom (n = 133)		Suspected case (n = 77)		Probable case (n = 38)		P-value*
		(n = 248)	n	(%)	n	(%)	n	(%)	
Department	Medicine	156	76	57.1	51	66.2	29	76.3	0.0075
	Nursing	92	57	42.9	26	33.8	9	23.7	
Grade	1	133	64	48.1	47	61.0	22	57.9	0.1652
	2	115	69	51.9	30	39.0	16	42.1	
Sex	Male	102	53	39.8	30	39.0	19	50.0	0.4784
	Female	146	80	60.2	47	61.0	19	50.0	
History	Allergic rhinitis	59	35	26.3	18	23.4	6	15.8	0.4032
	Anemia	30	15	11.3	9	11.7	6	15.8	0.7474
	Food Allergy	12	7	5.3	3	3.9	2	5.3	0.8979
	Heart disease	2	2	1.5	0	0.0	0	0.0	0.4182
	Liver disease	1	1	0.8	0	0.0	0	0.0	0.6479
	Renal disease	4	4	3.0	0	0.0	0	0.0	0.1724
	Diabetes	1	1	0.8	0	0.0	0	0.0	0.6479
	None	90	48	36.1	32	41.6	10	26.3	0.2778
Vaccination record for DTaP vaccine									
	No	3	1	0.8	1	1.3	1	2.6	0.0742
	1 shot	4	2	1.5	2	2.6	0	0.0	
	2 doses	2	0	0.0	0	0.0	2	5.3	
	3 doses	14	5	3.8	5	6.5	4	10.5	
	4 doses	225	125	94.0	69	89.6	31	81.6	

DTaP: diphtheria, tetanus, and pertussis.
*Chi-square test or Fisher's exact test.

Table 3 Relative risks of history of DTaP vaccination for pertussis according to case definition

Definition of pertussis	Number	Case	Attack rate (%)	Relative risk	(95% CI)	Relative risk[a]	(95% CI)
Probable cases							
Less than 4 doses or no doses	23	7	30.4	1		1	
4 doses	225	31	13.8	0.45	(0.23-0.91)	0.48	(0.24-0.97)
Probable + Suspected cases							
Less than 4 doses or no doses	23	15	65.2	1		1	
4 doses	225	100	44.4	0.68	(0.49-0.95)	0.70	(0.51-0.98)
Anti- PT antibody titers after outbreak ≥ 100EU/mL							
Less than 4 doses or no doses	23	2	8.7	1		1	
4 doses	225	13	5.8	0.64	(0.16-2.76)	0.64	(0.16-252)
Diagnosed as pertusis at medical institutions							
Less than 4 doses or no doses	23	2	8.7	1		1	
4 doses	225	17	7.6	0.87	(0.21-3.53)	0.74	(0.21-2.61)

DTaP: diptheria, tetanus, and pertussis; CI: confidence interval; PT: pertussis toxin.
Clinical criteria: (1) cough illness lasting ≧ 2 weeks; (2) paroxysms of coughing; (3) inspiratory "whoop"; (4) post-tussive vomiting.
Probable case: a patient who met cough illness lasting ≧ 2 weeks with at least 1 item in the other clinical criteria.
Suspected case: a patient who met at least 1 item in the above 4 clinical criteria.
[a]Adjusted by department using the Mantel-Haenszel method.

the outbreak. In addition, approximately 1 week of university holidays owing to consecutive holidays in May might also have reduced the spread of infection.

The vaccine effectiveness was 52% for probable cases meeting the clinical criteria for pertussis when students with fewer than 4 or no shots was defined as the reference. It is difficult to directly compare these results with other studies because booster vaccination recommendations vary by country [1,7,8,18], studies use different case definitions [1,18,19], vaccine effectiveness decreases with age-associated decreases in vaccine-induced antibodies [5,20,21], and study subject characteristics may also differ considerably between studies. We report a vaccine effectiveness lower than the 96% effectiveness reported by case–control studies of children in Japan with 3 or more vaccine doses compared to unvaccinated children [15], and about 80% reported by a meta-analysis study of children who received 4 vaccine doses [18]. Considering that the mean age in our study population was 20.4 years, the length of time since the last vaccination may contribute the lower vaccine effectiveness. This observation suggests that replacing the conventional diphtheria and tetanus toxin vaccine administered in adolescence to DTaP might be necessary in Japan. In addition, complete vaccination in infancy is essential, since incomplete vaccination did not show protective effects against pertussis in this study.

In other countries, the DTaP vaccine is administered in early childhood, and a Tdap booster vaccination is administered after early adolescence. Therefore, there are limited reports on the effectiveness of the DTaP vaccine in adolescents and adults. In a case–control study performed during an outbreak at a military school in France, the vaccine effectiveness rates among biologically confirmed cases where 5 and 4 DTaP vaccinations were administered was 32% and 22%, respectively [20]. This study also found that effectiveness decreased as the period from the last vaccination increased. On the other hand, 2 case–control studies in adolescents and adults after Tdap booster vaccination reported an effectiveness around 60%; these studies defined patients diagnosed with pertussis by PCR as cases and patients with pertussis-like symptoms but negative by PCR as controls [22,23]. In our study, the effectiveness of the DTaP vaccine was higher than that in a previous report from a US military school and slightly lower than that of Tdap effectiveness. However, because the vaccination series is completed by 2 years of age in Japan, 16 years or more had passed since the last vaccination. We also defined cases based on clinical criteria. Other reasons for these disparate results may be due to the effects of boosters administered during a pertussis outbreak in Japan in 2008 and 2009 [9-13]. Other reasons may include a higher rate of completed vaccine courses: in our study population, the vaccination coverage, or the percentage of the study population that had received 4 vaccine doses, was 94%. Differences in vaccine components [24,25] and vaccination methods (subcutaneous injection in Japan vs. intramuscular injection in the US) may also have contributed to differences in reported results.

Generally, the more precisely defined the outcome, the higher the diagnosis sensitivity [19], and detected effectiveness. In our study, the vaccine effectiveness against probable cases was higher than against suspected cases.

However, differences in vaccine effectiveness were not detected by serologically or medically diagnosed cases. In our study, patients with serum anti-PT antibody titers greater than 100 EU/mL at the end of July were considered positive for pertussis, although we could not perform examinations with paired sera to compare levels during the acute phase to the recovery phase of pertussis. Anti-PT antibodies have been reported to decrease particularly rapidly [26], so a patient with pertussis might not show as positive if antibody levels had fallen below this threshold. If many subjects with pertussis were not detected by serological testing, misclassification might occur. Medically diagnosed cases might be confounded by health-related behavior. Not all probable cases visited medical institutions; thus, outcomes were likely biased.

This study had several limitations. First, pertussis was diagnosed only on the basis of clinical criteria. The clinical definition of probable case includes a continuous cough for 14 days or more. In this study, promotion of active interventions and medical examinations at medical institutions occurred during the early phase of the outbreak for the purpose of infection control; as a result, the average cough duration decreased, leading to potential misclassification. Second, we could confirm vaccination records for only half of the study participants. Although misclassification of the vaccination category could be avoided by including only those participants whose maternity record books could confirm vaccination status, the sample size would be quite small. However, since the outbreak of pertussis occurred mainly in first and second grade students in our study, we believe that statistically significant differences in vaccine efficacy could be detected. Third, the past history of pertussis in the study participants is unknown. Since outbreaks of pertussis in high school students have also been recently reported in Japan [12], some students may have been infected with pertussis before entering the university. It is generally believed that a history of pertussis is protective against future pertussis owing to antibodies acquired by natural infection. Inclusion of subjects with a history of pertussis in the group that had not received 4 vaccinations in infancy could lead to underestimated vaccine effectiveness. Finally, hygiene behaviors might confound the association between the vaccination record and onset. For example, if those who did not receive recommended infant vaccinations were also not taught appropriate hygiene behaviors, they might not take prophylactic actions against infection, such as washing hands, wearing facemasks, and avoiding crowds during the pertussis season. However, if these differences exist, the effects are minimal, because vaccine effectiveness was not detected when we examined the association between measles vaccination with pertussis in the subjects of this study (Additional file 2: Table S2).

Conclusions

An outbreak of pertussis was observed in a population in which the majority of individuals had completed the DTaP vaccine course as infants. The AR was higher in students who did not complete the full infant DTaP vaccine course. The vaccine effectiveness was an estimated 52%, lower than that described in previous reports of children, mostly likely because of decreasing antibody levels in the long period of time since their last DTaP dose. These results suggest the necessity for booster vaccination for teenagers and development of highly efficacious pertussis vaccines.

Additional files

Additional file 1: Table S1. Characteristics of 248 students with Confirmed DTaP Vaccination History according to Completeness of Infant Vaccination.

Additional file 2: Table S2. Relative Risks for Pertussis by History of Measles Vaccination According to Case Definition in 248 Students with Confirmed Vaccination Records.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MH, KO, TN, SO, and YH designed the study. MH, MF, and IO participated in data collection. MH, KT, KO, TN, SO, WF and YH participated in data analysis and interpretation and wrote the report. All authors reviewed the data and approved the final report.

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Author details

[1]Department of Preventive Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga City, Saga 849-8501, Japan. [2]Department of Infection Control, Saga-ken Medical Centre Koseikan, 400 Nakahara, Kase, Saga City, Saga 840-8571, Japan. [3]Department of Pediatrics, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga City, Saga 849-8501, Japan. [4]Health Care Center, Saga University, 5-1-1 Nabeshima, Saga City, Saga 849-8501, Japan. [5]Department of Public Health, Faculty of Medicine, Osaka City University, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan. [6]Department of Pediatrics, Fukuoka Dental College, 2-15-1 Tamura, Sawara-ku, Fukuoka City, Fukuoka 814-0193, Japan. [7]Department of Pediatrics, Kawasaki Medical School, 577 Matsushima, Kurashiki City, Okayama 701-0192, Japan. [8]Clinical Epidemiology Research Center, Medical Co. LTA, 6-18, Ten-ya-machi, Hakata-ku, Fukuoka 812-0025, Japan.

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References

1. Zepp F, Heininger U, Mertsola J, Bernatowska E, Guiso N, Roord J, et al. Rationale for pertussis booster vaccination throughout life in Europe. Lancet Infect Dis. 2011;11(7):557–70.
2. Cherry JD. The present and future control of pertussis. Clin Infect Dis. 2010;51(6):663–7.
3. Cherry JD. Epidemic pertussis in 2012–the resurgence of a vaccine-preventable disease. N Engl J Med. 2012;367(9):785–7.
4. Cherry JD. Why do pertussis vaccines fail? Pediatrics. 2012;129(5):968–70.

5. Wendelboe AM, Van Rie A, Salmaso S, Englund JA. Duration of immunity against pertussis after natural infection or vaccination. Pediatr Infect Dis J. 2005;24(5 Suppl):S58–61.
6. Clark TA. Changing pertussis epidemiology: everything old is new again. J Infect Dis. 2014;209(7):978–81.
7. Bechini A, Tiscione E, Boccalini S, Levi M, Bonanni P. Acellular pertussis vaccine use in risk groups (adolescents, pregnant women, newborns and health care workers): a review of evidences and recommendations. Vaccine. 2012;30(35):5179–90.
8. CDC. Updated Recommendations for Use of Tetanus Toxoid, Reduced Diphtheria Toxoid, and Acellular Pertussis (Tdap) Vaccine in Adults Aged 65 Years and Older —Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2012;61:468–70.
9. Han HJ, Kamachi K, Okada K, Toyoizumi-Ajisaka H, Sasaki Y, Arakawa Y. Antigenic variation in Bordetella pertussis isolates recovered from adults and children in Japan. Vaccine. 2008;26(12):1530–4.
10. Kamano H, Mori T, Maeta H, Taminato T, Ishida T, Kishimoto N, et al. Analysis of Bordetella pertussis agglutinin titers during an outbreak of pertussis at a university in Japan. Jpn J Infect Dis. 2010;63(2):108–12.
11. Miyashita N, Kawai Y, Yamaguchi T, Ouchi K, Kurose K, Oka M. Outbreak of pertussis in a university laboratory. Intern Med. 2011;50(8):879–85.
12. Kamiya H, Otsuka N, Ando Y, Odaira F, Yoshino S, Kawano K, et al. Transmission of Bordetella holmesii during pertussis outbreak, Japan. Emerg Infect Dis. 2012;18(7):1166–9.
13. Otsuka N, Han HJ, Toyoizumi-Ajisaka H, Nakamura Y, Arakawa Y, Shibayama K, et al. Prevalence and genetic characterization of pertactin-deficient Bordetella pertussis in Japan. PLoS One. 2012;7(2):e31985.
14. Saito T, Akamatsu T, Aoyama T, Iwai H, Gonda T, Murase Y, et al. [Efficacy of acellular pertussis vaccine] in Japanese. Kansenshogaku Zasshi. 1990;64(5):564–9.
15. Okada K, Ohashi Y, Matsuo F, Uno S, Soh M, Nishima S. Effectiveness of an acellular pertussis vaccine in Japanese children during a non-epidemic period: a matched case–control study. Epidemiol Infect. 2009;137(1):124–30.
16. Kamachi K, Toyoizumi-Ajisaka H, Toda K, Soeung SC, Sarath S, Nareth Y, et al. Development and evaluation of a loop-mediated isothermal amplification method for rapid diagnosis of Bordetella pertussis infection. J Clin Microbiol. 2006;44(5):1899–902.
17. Centers for Disease Control and Prevention. National Notifiable Diseases Surveillance System. Pertussis (Whooping Cough) (bordetella pertussis) 2014 case definition. 2014. Available from: http://wwwn.cdc.gov/nndss/script/casedef.aspx?CondYrID=950&DatePub=1/1/2014. Accessed January 30, 2015.
18. Jefferson T, Rudin M, DiPietrantonj C. Systematic review of the effects of pertussis vaccines in children. Vaccine. 2003;21(17–18):2003–14.
19. Cherry JD, Tan T, Wirsing von Konig CH, Forsyth KD, Thisyakorn U, Greenberg D, et al. Clinical definitions of pertussis: summary of a global pertussis initiative roundtable meeting, February 2011. Clin Infect Dis. 2012;54(12):1756–64.
20. Berger F, Njamkepo E, Minaberry S, Mayet A, Haus-Cheymol R, Verret C, et al. Investigation on a pertussis outbreak in a military school: risk factors and approach to vaccine efficacy. Vaccine. 2010;28(32):5147–52.
21. Klein NP, Bartlett J, Rowhani-Rahbar A, Fireman B, Baxter R. Waning protection after fifth dose of acellular pertussis vaccine in children. N Engl J Med. 2012;367(11):1012–9.
22. Wei SC, Tatti K, Cushing K, Rosen J, Brown K, Cassiday P, et al. Effectiveness of adolescent and adult tetanus, reduced-dose diphtheria, and acellular pertussis vaccine against pertussis. Clin Infect Dis. 2010;51(3):315–21.
23. Baxter R, Bartlett J, Rowhani-Rahbar A, Fireman B, Klein NP. Effectiveness of pertussis vaccines for adolescents and adults: case–control study. BMJ. 2013;347:f4249.
24. Okada K, Komiya T, Yamamoto A, Takahashi M, Kamachi K, Nakano T, et al. Safe and effective booster immunization using DTaP in teenagers. Vaccine. 2010;28(48):7626–33.
25. Hara M, Okada K, Yamaguchi Y, Uno S, Otsuka Y, Shimanoe C, et al. Immunogenicity and safety after booster vaccination of diphtheria, tetanus, and acellular pertussis in young adults: an open randomized controlled trial in Japan. Clin Vaccine Immunol. 2013;20(12):1799–804.
26. Ward JI, Cherry JD, Chang SJ, Partridge S, Lee H, Treanor J, et al. Efficacy of an acellular pertussis vaccine among adolescents and adults. N Engl J Med. 2005;353(15):1555–63.

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RESEARCH ARTICLE

Economic Evaluation of Immunisation Programme of 23-Valent Pneumococcal Polysaccharide Vaccine and the Inclusion of 13-Valent Pneumococcal Conjugate Vaccine in the List for Single-Dose Subsidy to the Elderly in Japan

Shu-ling Hoshi[1]*, Masahide Kondo[2], Ichiro Okubo[3]

1 Department of Health Care Policy and Health Economics, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, **2** Department of Health Care Policy and Health Economics, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, **3** Department of Health Care Policy and Health Economics, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

* hoshi@hcs.tsukuba.ac.jp

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Abstract

Background

Currently in Japan, both 23-valent pneumococcal polysaccharide vaccine (PPSV–23) and 13-valent pneumococcal conjugate vaccine (PCV–13) are available for the elderly for the prevention of *S. pneumoniae*-related diseases. PPSV–23 was approved in 1988, while the extended use of PCV–13 was approved for adults aged 65 and older in June 2014. Despite these two vaccines being available, the recently launched national immunisation programme for the elderly only subsidised PPSV–23. The framework of the current immunisation programme lasts for five years. The elderly population eligible for the subsidised PPSV–23 shot for the 1st year are those aged 65, 70, 75, 80, 85, 90, 95 and ≥100. While from the 2nd year to the 5th year, those who will age 65, 70, 75, 80, 85, 90, 95 and 100 will receive the same subsidised shot.

Methods

We performed economic evaluations to (1) evaluate the efficiency of alternative strategies of PPSV–23 single-dose immunisation programme, and (2) investigate the efficiency of PCV–13 inclusion in the list for single-dose pneumococcal vaccine immunisation programme. Three alternative strategies were created in this study, namely: (1) current PPSV–23 strategy, (2) 65 to 80 (as "65–80 PPSV–23 strategy"), and (3) 65 and older (as "≥65 PPSV–23 strategy"). We constructed a Markov model depicting the *S. pneumoniae*-related disease course pathways. The transition probabilities, utility weights to estimate quality adjusted life year (QALY) and disease treatment costs were either calculated or cited from

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literature. Cost of per shot of vaccine was ¥8,116 (US$74; US$1 = ¥110) for PPSV–23 and ¥10,776 (US$98) for PCV–13. The model runs for 15 years with one year cycle after immunisation. Discounting was at 3%.

Results

Compared to current PPSV–23 strategy, 65–80 PPSV–23 strategy cost less but gained less, while the incremental cost-effectiveness ratios (ICERs) of ≥65 PPSV–23 strategy was ¥5,025,000 (US$45,682) per QALY gained. PCV–13 inclusion into the list for single-dose subsidy has an ICER of ¥377,000 (US$3,427) per QALY gained regardless of the PCV–13 diffusion level. These ICERs were found to be cost-effective since they are lower than the suggested criterion by WHO of three times GDP (¥11,000,000 or US$113,636 per QALY gained), which is the benchmark used in judging the cost-effectiveness of an immunisation programmne.

Conclusions

The results suggest that switching current PPSV–23 strategy to ≥65 PPSV–23 strategy or including PCV–13 into the list for single-dose subsidy to the elderly in Japan has value for money.

Introduction

23-valent pneumococcal polysaccharide vaccine (PPSV–23) has been recommended for prevention of invasive pneumococcal disease (IPD) in adults since 1983 [1]. It was the only pneumococcal vaccine available for all adults aged 65 and older until the approval of the extended use of 13-valent pneumococcal conjugate vaccine (PCV–13) for prevention of pneumococcal pneumonia and IPD in adults 50 years and older on December 30, 2011 by US Food and Drug Administration [1]. On August 13, 2014, the Advisory Committee on Immunization Practices of US Centers for Disease Control and Prevention modified the recommendation on pneumococcal vaccine for the elderly. The new recommendation states that *"Both PCV13 and PPSV23 should be routinely administered in series to all adults aged ≥65 years"*, which is based on the results of a randomised placebo-controlled trial showing PCV–13 efficacy against community-acquired pneumonia (CAP) among approximately 85,000 adults aged 65 and older [1]. PPSV–23 and PCV–13 differ in cost, number of serotypes covered, mechanism for immunogenicity, and level of effectiveness, particularly against non-bacteremic pneumococcal pneumonia (NPP).

Currently, in Japan, both PPSV–23 and PCV–13 are available for the elderly for the prevention of *S. pneumoniae*-related diseases. PPSV–23 was approved in 1988 [2]. However, only some municipalities coordinated publicly-funded pneumococcal immunisation programmes for the elderly from 2001 through September 2014; vaccine coverage was about 25% [3]. On the other hand, the extended use of PCV–13 in adults aged 65 and older was approved in June 2014. Despite of these two vaccines being available for elderly, the national immunisation programme launched for the elderly aged 65 and older on October 1, 2014 only subsidised PPSV–23. The framework of the current immunisation program lasts for five years. The elderly population eligible for the subsidised PPSV–23 shot for the 1st year are those aged 65, 70, 75, 80, 85, 90, 95 and ≥100. While from the 2nd year to the 5th year, those who will age 65, 70, 75, 80, 85,

90, 95 and 100 will receive the same subsidised shot [4]. Countries, where publicly funded PPSV–23 immunisation programmes for elderly have been launched, set the eligible age to receive a shot of subsidised vaccine as 65 to 80, 65 and older, 70 and older, and so on [1, 5–9]. Due to the limited resources for health care, there is a need to organize an efficient immunisation programme. This study builds upon such need and intends to address such issues by (1) evaluating the efficiency of alternative strategies of PPSV–23 immunisation programmes, and (2) investigating the efficiency of PCV–13 inclusion in the list of single-dose pneumococcal vaccine national immunisation programme.

Methods

We conducted a cost-effectiveness analysis with Markov modelling from payers' perspective. We conducted a literature survey to define the alternative immunisation programmes and to construct the model. Studies pertaining to epidemiology and prognosis of relevant diseases caused by *S. pneumoniae* in Japan's setting were accessed from PubMed database, Igaku Chuo Zasshi (Japana Centra Revuo Medicina) database, Ministry of Health, Labour and Welfare (MHLW) Grant System, and annual statistic reports published by the government. Igaku Chuo Zasshi, a Japanese medical bibliographic database, which contains over 10 million citations originating from Japan, comprehensively covers articles published in Japanese-language medical journals. Due to insufficient evidences from Japan, overseas' reports from PubMed, Medline, The Cochrane Database of Systematic Reviews, Health Technology Assessment database, and The NHS Economic Evaluation Database regarding vaccine effectiveness, utility weights to estimate quality adjusted life year (QALY) and economic evaluation related to vaccines were used instead.

PPSV–23 programmes and inclusion of PCV–13

The target population of the immunisation programmes to be evaluated are those aged 65 and older in 2014. In evaluating the efficiency of different PPSV–23 immunisation programmes, we set three different strategies with different ages to receive a shot of subsidised vaccine, namely: (1) current PPSV–23 strategy, (2) 65 to 80 (as "65–80 PPSV–23 strategy"), and (3) 65 and older (as "≥65 PPSV–23 strategy"). Age-specific populations were from demographic data [10]. Current PPSV–23 strategy served as a comparator of the other two strategies. In 65–80 PPSV–23 strategy, those who aged over 80 were not eligible to the immunisation programme, which means these individuals will only follow the transition probabilities assigned to the corresponding ages without any vaccine effectiveness on reducing any *S. pneumoniae*-related diseases. Vaccine uptake rates were assumed at 50.4% for all strategies, which was the same with the coverage rate of seasonal influenza vaccine in 2013 [11].

In order to investigate the cost-effectiveness of PCV–13 inclusion in the list for single-dose pneumococcal vaccine national immunisation programme, we made variations on the share of PCV–13 between the two pneumococcal vaccines from 10% to 100% with 10% interval, because it is unknown how doctors, vaccinees, and municipalities will choose between PPSV–23 and PCV–13. Ten levels of diffusion of PCV–13 were compared with current PPSV–23 strategy.

Only single-dose subsidy was analysed and not the sequence of PCV–13/PPSV–23 or PPSV–23/PCV–13, this is mainly due to PPSV–23 immunisation being a newly-launched programme in Japan [12, 13]. We reserve the evaluation of the cost-effectiveness of uptaking the two vaccines in the future research so as to delineate and emphasize on the main purpose of the study.

Markov model

A Markov model of courses followed by the cohort under consideration was constructed based on epidemiological data, vaccine effectiveness and models from previous studies. Seven mutually-exclusive health states were modelled: health (without any *S. pneumoniae*-related diseases), bacteremia without pneumococcal pneumonia, bacteremia with pneumococcal pneumonia, meningitis, CAP caused by *S. pneumoniae*, neurological sequelae and death of or other than the related diseases (Fig 1). A Markov cycle for each stage was set at one year with a cohort timeframe of 15 years after being vaccinated. We assumed all the individuals who survived until the timeframe age have a life expectancy of the Japanese population [14]. Adverse effects associated with vaccination of PPSV–23 and PCV–13 were not considered, since they were mild or moderate in severity [15–17]. Considering that all transition states did not occur simultaneously at the end of each cycle, while in reality, most kinds of transitions typically occur gradually throughout a time interval (on average, half-way through), we implemented a half-cycle correction in estimating the incremental cost-effectiveness ratios (ICERs) of the programmes [18]. The half-cycle correction is implemented by using one-half of every state's incremental reward in model's initial and final reward.

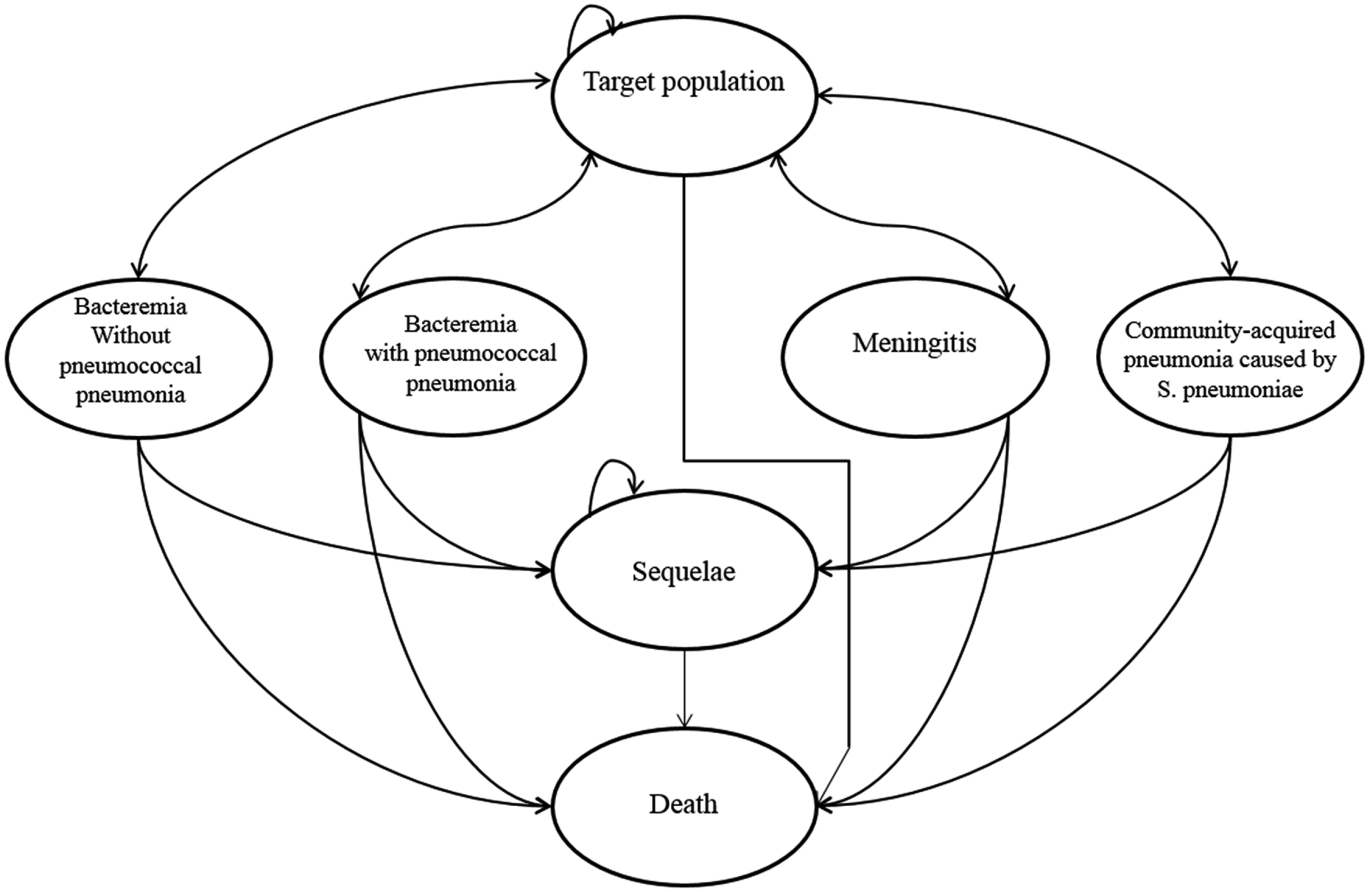

Fig 1. Markov Model.

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Outcomes estimation

Outcomes in terms of QALY were estimated by assigning transition probabilities and utility weights from literature. We estimated the 5-year age-specific incidence rates using the following: (1) annual IPD incidence rates among persons age 65 and over (2.41 per 100,000) [19], (2) IPD distribution by age [19], and (3) demographic data [10]. NPP annual incidence rates were estimated as incidence of CAP times proportion of *S. pneumonia*-caused CAP at 17.2% [20]. CAP incidence rates, 10.7 and 42.9 per 1000 person-years for persons who were aged 65–74 and aged ≥75, respectively, were from a 3-year prospective hospital-based surveillance [21]. Proportions of bacteremia with/without pneumococcal pneumonia, and meningitis among IPD cases were from the Infectious Agents Surveillance Report (IASR) [19]. Ubukata's results of IPD case-fatality rates and proportion that results in neurological sequelae among IPD cases and NPP cases were used in the study [22]. NPP case-fatality rate was from Ishida et al.'s study, which reported the rate among patients with positive urinary antigen test of *S. pneumoniae*-related pneumonia [23]. Deaths of causes other than the above diseases were taken from the vital statistics [24]. Utility weights used to calculate QALY were assumed based on a study by Smith et al. [25]. Average lengths of hospital stay were from published government data [26]. All these data are shown in Table 1.

Vaccine effectiveness of PPSV–23 in reducing IPD incidence rate was cited from a Cochrane Review report [30]. Results from meta-analysis show that the use of PPSV–23 to prevent vaccine serotype IPD in adults of high-income countries, was at 82% (69%-90%), while its effectiveness in reducing non-IPD was inconsistent. We assumed that the effectiveness in reducing non-IPD to be 0% [30]. PCV–13 effectiveness in reducing vaccine-serotype IPD and non-invasive vaccine-type CAP, 75.0% and 45.0%, respectively, were from a randomised placebo-controlled trial study [31]. We assumed the effectiveness of both vaccines reduce by age of vaccination and by years after being vaccinated. Extent of reduction was proportional to the effectiveness used in Smith et al.'s study [25]. All these data are shown in Table 2. The vaccine serotypes causing IPD among elderly were 60.0% and 46.0% for PPSV–23 and PCV–13 [19], respectively, those for NPP were 62.7% and 49.3% [13] (Table 1).

Costing

In this study, costs borne by government, municipalities, vaccinees, patients and third party payers were considered, while advertising costs borne by manufacturers were left unaccounted. It is obvious that when a new product enters a market, which was monopolised by the other product, the manufacturers of both products will invest a lot to compete for the share in the market. Since the decision maker, MHLW Vaccine Committee, was only interested in costs borne by the aforementioned payers, we omitted the inclusion of this cost. Non-direct medical costs related to the immunisation programme were not included, because the programme was built within the public health services routine [32]. The amount of direct payments to health care providers by these entities was estimated as costs. Cost items were identified along the decision tree and Markov model. We used the literature along with some assumptions to estimate necessary data.

Age-specific treatment costs of per case of bacteremia with/without pneumococcal pneumonia and pneumonia were estimated from Status Survey on Medical Care Benefits [26]. Cost per case of meningitis was assumed to be twice the cost per case of bacteremia, while cost of sequelae was assumed ¥1,500,000 (US$13,636) per case per year [27]. One vaccine shot was assumed at ¥8,116 (US$ 74: US$1 = ¥110) for PPSV–23 and ¥10,776 (US$98) for PCV–13, which were the sum of vaccine price (¥4,737 or US$43 for PPSV–23, ¥7,200 or US$65 for

Table 1. Model inputs.

Distribution of population among adults aged 65 and older									
Age	**%**								[10]
65, 66, 67, 68, 69, 70, 71, 72, 73, 74,	6.9, 6.6, 4.1, 4.4, 5.3, 5.1, 5.2, 5.0, 4.5, 3.9,								
75, 76, 77, 78, 79, 80, 81, 82, 83, 84,	4.1, 4.2, 4.1, 3.8, 3.5, 3.4, 3.2, 3.0, 2.7, 2.5,								
85, 86, 87, 88, 89, 90, 91, 92, 93, 94,	2.3, 2.1, 1.9, 1.6, 1.3, 1.1, 0.9, 0.7, 0.6, 0.4,								
95, 96, 97, 98, 99,100+	0.3, 0.3, 0.2, 0.1, 0.1, 0.2								
	Percentage of female among each age								
	51.4, 51.5, 51.9, 52.4,52.7, 52.9,53.2, 53.4, 53.8, 54.2,								
	54.8, 55.3,55.9,56.7,57.6, 58.6, 59.3, 60.4,61.5,62.8								
	63.9, 65.4, 66.9, 69.2, 71.2 74.4, 76.3, 77.7, 78.4, 79.9								
	81.1, 81.6, 83.6,84.1,84.8, 87.3								
Rate and proportions[a]	**65+**	**65–69**	**70–74**	**75–79**	**80–84**	**85–89**	**90–94**	**95+**	
Annual incidence rate of IPD (per 100,000 population)	2.4	1.8	1.8	2.5	2.7	4.2	4.4	4.2	[10, 19]
Bacteremia without pneumococcal pneumonia among IPD cases (%)	35.6	36.8	40.0	29.8	38.0	33.9	42.1	16.7	[19]
Bacteremia with pneumococcal pneumonia among IPD cases (%)	45.8	39.5	35.5	50.7	45.1	54.8	50.0	66.7	[18]
Meningitis among IPD cases (%)	18.6	23.6	24.5	19.6	16.8	11.3	7.9	16.7	[19]
Annual incidence rate of CAP (per 1,000 population)	10.7 (aged 65–74), 42.9 (age > = 75)								[21]
CAP caused by *S. pneumoniae* (%)	17.2								[20]
Bacteremia without pneumococcal pneumonia result in sequelae (%)	2.0								[22]
Bacteremia with pneumococcal pneumonia result in sequelae (%)	7.0								[22]
Meningitis result in sequelae (%)	30.0								[22]
Non-bacteremic pneumococcal pneumonia result in sequelae (%)	2.7								[22]
Case-fatality rate (%)									
Bacteremia without pneumococcal pneumonia	25.0								[22]
Bacteremia with pneumococcal pneumonia	30.5								[22]
Meningitis	14.9								[22]
Non-bacteremic pneumococcal pneumonia	1.9								[23]
Sequelae	5.0								[22]
Serotypes covering of disease caused by *S. pneumococcus*									
Invasive pneumococcal diseases	PPSV–23: 60.0%		PCV–13: 46.0%						[19]
Non-bacteremic pneumococcal pneumonia	PPSV–23: 62.9%		PCV–13: 49.3%						[13]
Utility weights									[25]
Health	1								
Bacteremia without pneumococcal pneumonia	0.5								
Bacteremia with pneumococcal pneumonia	0.5								
Meningitis	0.4								
Pneumococcal pneumonia	0.5								
Sequelae	0.3								
Death	0								
Average lengths of hospital stay (day)	**65–69**	**70–74**	**75–79**	**80–84**	**85–89**	**90–94**	**95–99**	**100+**	
Bacteremia/pneumonia	12.3	13.1	14.1	14.9	15.6	16.1	16.3	16.2	[26]
Meningitis	24.5	26.3	28.3	29.9	31.2	32.1	32.6	32.4	[26]
Treatment costs per case (¥)									

(*Continued*)

Table 1. (*Continued*)

Distribution of population among adults aged 65 and older									
Bacteremia/pneumonia	428,005	440,028	453,172	453,404	449,147	435,079	425,829	408,372	[26]
Meningitis	856,011	880,057	906,343	906,808	898,293	870,158	851,658	816,744	[26]
Sequelae (per case per year)	1,500,000								[27]
Costs per vaccination (¥)									
Cost per PPSV–23 shot	8,116								[28, 29]
Cost per PCV–13 shot	10,776								[28, 29]

[a]On Markov model, transition probabilities from health state A to health state B by ages were calculated as follows

From "Health" to "Bacteremia without pneumococcal pneumonia" = Annual incidence rate of IPD × Bacteremia without pneumococcal pneumonia among IPD cases

From "Health" to "Bacteremia with pneumococcal pneumonia" = Annual incidence rate of IPD × Bacteremia with pneumococcal pneumonia among IPD cases

From "Health" to "Meningitis" = Annual incidence rate of IPD × Meningitis among IPD cases

From "Health" to "Non-bacteremic pneumococcal pneumonia" = Annual incidence rate of CAP × CAP caused by *S. pneumoniae*

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PCV–13) [28, 29], doctor fee and technical fee for PPSV–23 and PCV–13, respectively. All cost data are shown in Table 1.

Discounting

Outcomes and costs were discounted at a rate of 3% [32].

One-way sensitivity analyses and probabilistic analyses

We performed one-way sensitivity analyses on two pairs of comparisons. The first pair is ≥65 PPSV–23 strategy vs. current PPSV–23 strategy, which is to appraise the stability of ICERs with the assumptions made in our economic model, and to explore the impact of each variable relative to each other when the subsidy of the immunisation is limited to PPSV–23. The second pair is PCV–13 strategy vs. current PPSV–23 strategy, which is to appraise the same issues when PCV–13 is also subsidised by current immunisation programme. The lower limits and upper limits used in sensitivity analyses were ±50% for costs variables and ±20% for probabilities and utilities. We also conducted two sets of 1000 Monte Carlo simulations on ≥65 PPSV–23 strategy and 65–80 PPSV–23 strategy vs. current PPSV–23 strategy, i.e., probabilistic analyses, for which all data were assumed to have an equilateral triangle distribution corresponding to the range tested in one way sensitivity analyses. Triangular distribution was used because of the insufficiency of information about distributions. This distribution has been theoretically proven as both simple and efficient, which can serve as a proxy for beta or other distributions in risk analysis [33–35].

Results

Costs, effectiveness, and cost-effectiveness of alternative PPSV–23 single-dose immunisation strategies

Compared to current PPSV–23 strategy, the incremental cost and incremental effectiveness per person for ≥65 PPSV–23 strategy were ¥216 (US$2) and 0.00004 QALYs; estimated ICER

Table 2. Data used to estimate vaccine effectiveness (VE) and VEs used in the model.

Data used to estimate vaccine effectiveness (1–3)									
1. Vaccine effectiveness of PPSV–23 and PCV–13 in preventing IPD used in Smith et. al's study [25] (%)									
	PPSV–23						**PCV–13**		
years post	**aged 65–79**			**aged 80 and over**			**aged 65 and over**		
vaccination	**base-case**	**low**	**high**	**base-case**	**low**	**high**	**base-case**	**low**	**high**
1	80.0	60.0	90.0	67.0	20.0	85.0	85.0	60.0	95.0
3	73.0	50.0	83.0	53.0	0	83.5	80.0	45.0	90.0
5	58.0	30.5	80.0	32.0	0	75.0	70.0	30.0	87.0
7	33.0	13.0	48.0	10.0	0	30.0	60.0	22.5	77.5
10	0	0	10.0	0	0	10.0	50.0	15.0	68.0
15	0	0	10.0	0	0	10.0	33.0	0	60.0
2. Vaccine effectiveness of PPSV–23 in preventing vaccine type IPD (in high income countries) (%)									
			(based on Cochrane database of systemic review [30])						
	82	69	90						
3. Vaccine effectiveness of PCV–13 in preventing IPD and non-bacteremic CAP based on CApiTA study [31]) (%)									
	Reduced non-bacteremic vaccine type CAP			45.0					
	Reduced vaccine-type IPD			75.0					
VE in preventing IPD used in current study (%) (Based on 1, 2, and 3)									
	PPSV–23						**PCV–13**		
years post	**aged 65–79**			**aged 80 and over**			**aged 65–79**		
vaccination	**base-case**	**low**	**high**	**base-case**	**low**	**high**	**base-case**	**low**	**high**
1	82.0	69.0	90.0	68.7	23.0	85.0	75.0	52.9	83.8
3	74.8	57.5	83.0	54.3	0.0	83.5	70.6	39.7	79.4
5	59.5	35.1	80.0	32.8	0.0	75.0	61.8	26.5	76.8
7	33.8	15.0	48.0	10.3	0.0	30.0	52.9	19.9	68.4
10	0	0	10.0	0	0	10.0	44.1	13.2	60.0
15	0	0	10.0	0	0	10.0	29.1	0	52.9
VE in preventing non-invasive vaccine type CAP used in current study (%) (Based on 1, 2, and 3)									
	PPSV–23						**PCV–13**		
years post	**aged 65–79**			**aged 80 and over**			**aged 65–79**		
vaccination	**base-case**	**low**	**high**	**base-case**	**low**	**high**	**base-case**	**low**	**high**
1	-	-	-	-	-	-	45.0	31.8	50.3
3	-	-	-	-	-	-	42.4	23.8	47.6
5	-	-	-	-	-	-	37.1	15.9	46.1
7	-	-	-	-	-	-	31.8	11.9	41.0
10	-	-	-	-	-	-	26.5	7.9	36.0
15	-	-	-	-	-	-	17.5	0	31.8

aged 80 and over						
base-case	**low**	**high**	**base-case**	**low**	**high**	
62.8	17.6	79.2				
51.2	13.2	75.0				
34.1	8.8	72.5				
16.0	6.6	64.6				
0	0	56.7				
0	0	50.0				
aged 80 and over						
base-case	**low**	**high**				
37.7	26.6	42.1				
30.7	17.3	34.6				
20.4	8.8	25.4				
9.6	3.6	12.4				
0	0	0				
0	0	0				

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was ¥5,025,000 (US$45,682) per QALY gained. For 65–80 PPSV–23 strategy, incremental cost and incremental effectiveness were both negative values, which indicated that 60–80 PPSV–23 strategy cost less but also gained less than current PPSV–23 strategy (Table 3).

Costs, effectiveness, and cost-effectiveness of including PCV–13 in the list of single-dose subsidy

Among 10 scenarios with different PCV–13 diffusion levels, scenarios with higher PCV–13 diffusion level resulted in larger vaccine cost, while it saved more treatment costs and gained more QALYs compared to current scenario. Reduced treatment costs did not offset vaccination

Table 3. Cost, effectiveness and incremental cost-effectiveness ratio (vs. current scenario) by using PPSV–23 only.

	Vaccine cost per person	Treatment cost per person	Total cost per person	Effectiveness per person	Incremental cost	Incremental effectiveness	ICER* = (5)/(6)
	¥	¥	¥	QALY	¥	QALY	
	(1)	(2)	(3) = (1)+(2)	(4)	(5)	(6)	
Current strategy	3,860	20,456	24,316	14.31480	-	-	-
65–80 strategy	2,259	20,460	22,719	14.31480	-1,597	-0.00001	cost less, gain less
≥65 strategy	4,091	20,441	24,532	14.31485	216	0.00004	5025,000

*ICER: incremental cost-effectiveness ratio (¥/QALY gained). All ICERs were rounded to the nearest thousand.

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cost, which means gained more QALYs with more costs. ICERs were ¥378,000 (US$3,436) per QALY gained regardless of PCV–13 diffusion level (Table 4).

Results of one-way sensitivity analyses and probabilistic analyses

In one-way sensitivity analyses of ≥65 PPSV–23 strategy vs. current PPSV–23 strategy, the variables which were found to increase/decrease ICER more than ¥1,000,000 (US$9,091) are as follows: (1) cost of per vaccine shot, (2) IPD incidence rate, (3) vaccine effectiveness of PPSV–23 in reducing IPD incidence rate, and (4) percentage of vaccine serotype causing IPD (Fig 2A). In PCV–13 strategy vs. current PPSV–23 strategy, only costs per shot of PCV–13 and per shot of PPSV–23 were found to produce large changes in ICERs (Fig 2B). Figs 3 and 4 show the results of probabilistic analyses. Each dot on Fig 3 represents the incremental cost and effect obtained from one simulation following the random draw of model parameters from distribution. The cost-effectiveness acceptability curve (CEAC) shows that in ≥65 PPSV–23 strategy vs. current PPSV–23 strategy, among 1000 ICERs produced by Monte Carlo simulations, 61.5% are under ¥5,500,000 (US$50,000) per QALY and 100% are under ¥10,000,000 (US $90,910) per QALY (Fig 4).

Table 4. Cost, effectiveness and incremental cost-effectiveness ratio of different diffusion levels of PCV–13 (vs. current PPSV–23 strategy).

Diffusion level of PCV13 vs. PPSV–23	Vaccine cost per person	Treatment cost per person	Total cost per person	Effectiveness per person	Incremental cost	Incremental effectiveness	ICER* = (5)/(6)
	¥	¥	¥	QALY	¥	QALY	
	(1)	(2)	(3) = (1)+(2)	(4)	(5)	(6)	
0% vs. 100% (Current strategy)	3,860	20,456	24,316	14.31480	-	-	-
10% vs. 90%	3,987	20,350	24,337	14.31486	21	0.00006	378,000
20% vs. 80%	4,113	20,245	24,358	14.31491	42	0.00011	378,000
30% vs. 70%	4,240	20,140	24,380	14.31497	64	0.00017	378,000
40% vs. 60%	4,366	20,035	24,401	14.31503	85	0.00022	378,000
50% vs. 50%	4,493	19,929	24,422	14.31508	106	0.00028	378,000
60% vs. 40%	4,619	19,824	24,443	14.31514	127	0.00034	378,000
70% vs. 30%	4,746	19,719	24,464	14.31520	149	0.00039	378,000
80% vs. 20%	4,872	19,613	24,486	14.31525	170	0.00045	378,000
90% vs. 10%	4,999	19,508	24,507	14.31531	191	0.00051	378,000

*ICER: incremental cost-effectiveness ratio (¥/QALY gained). All ICERs were rounded to the nearest thousand.

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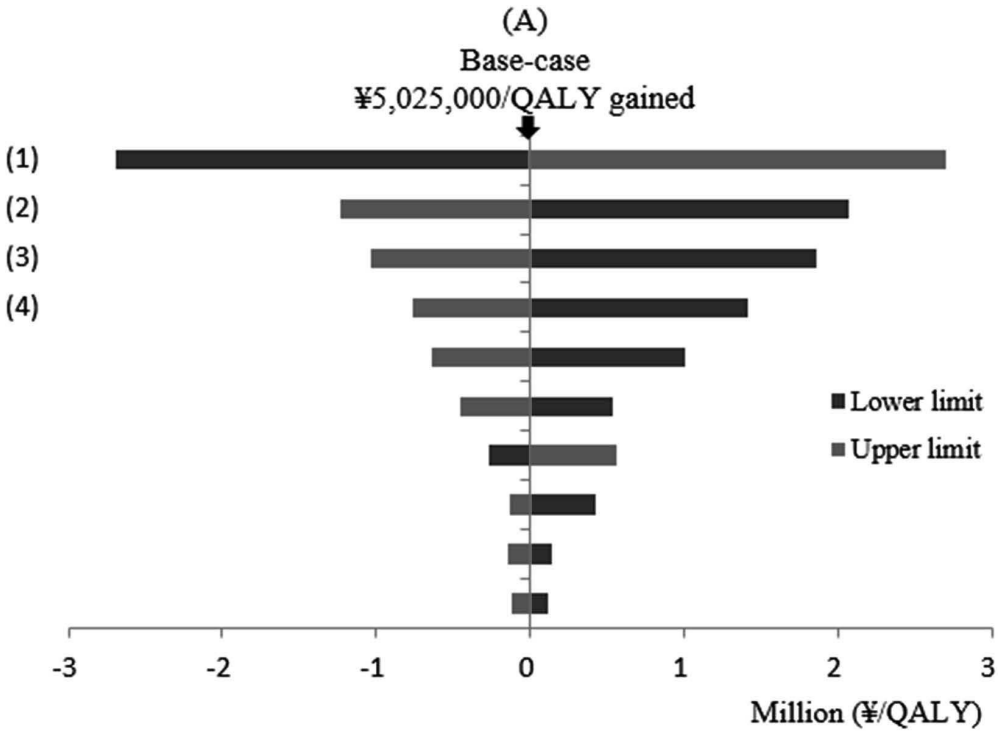

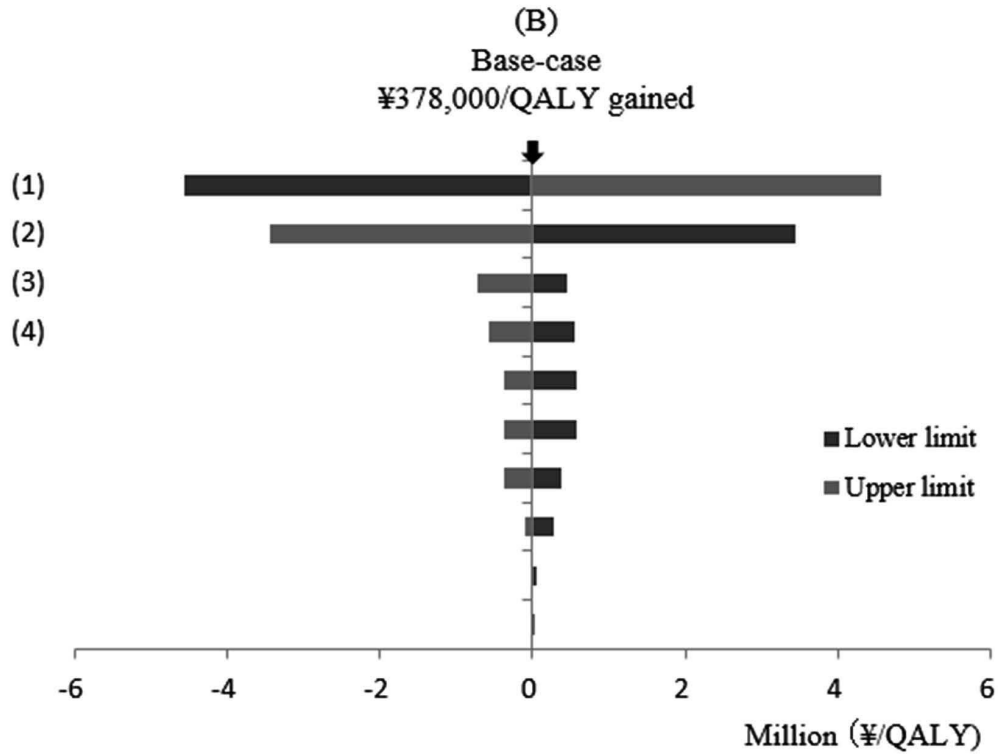

Fig 2. Results of one-way sensitivity analyses. (A) ≥65 PPSV–23 strategy vs. current PPSV–23 strategy. (B) PCV–13 strategy vs. current PPSV–23 strategy. In Fig 2A: (1) Cost per shot of PPSV–23, (2) Annual incidence rate of IPD, (3) Vaccine effectiveness of PPSV–23 in reducing IPD incidence rate, (4) Percentage of vaccine serotype causing IPD. In Fig 2-B: (1) Cost per shot of PCV–13. (2) Cost per shot of PPSV–23, (3) Vaccine effectiveness of PCV–13 in preventing noninvasive vaccine type CAP, (4) Treatment cost per *S. pneumoniae*-related case.

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Discussion

This study intends to address the following objectives: (1) to evaluate the efficiency of alternative PPSV–23 immunisation strategies compared to current PPSV–23 strategy, and (2) to investigate the cost-effectiveness of the inclusion of PCV–13 in the list of single-dose current pneumococcal vaccine national immunisation programme.

Compared to the current PPSV–23 strategy, incremental cost and incremental effectiveness of 65–80 PPSV–23 strategy were both negative, which means that switching current strategy to 65–80 strategy was found to gain less QALYs than current PPSV–23 strategy (this outcome will not be considered in decision-making). Switching current PPSV–23 strategy to ≥65 PPSV–23 strategy was found to be favourable (ICER at ¥5,025,000 or US$45,682 per QALY gained) compared to either of the suggested criterion by WHO of three times GDP (around ¥11,000,000 or US$113,636 per QALY gained in Japan) [36], or by Shiroiwa at ¥5,000,000 (US $45,455) per QALY gained [37]. Moreover, the result of probabilistic sensitivity analyses on switching current PPSV–23 strategy to ≥65 PPSV–23 strategy, ICER to be under ¥5,500,000 (US$50,000) per QALY is 61.5% and is 100% to be under ¥10,000,000 (US$90,910) per QALY gained, is deemed to be cost-effective.

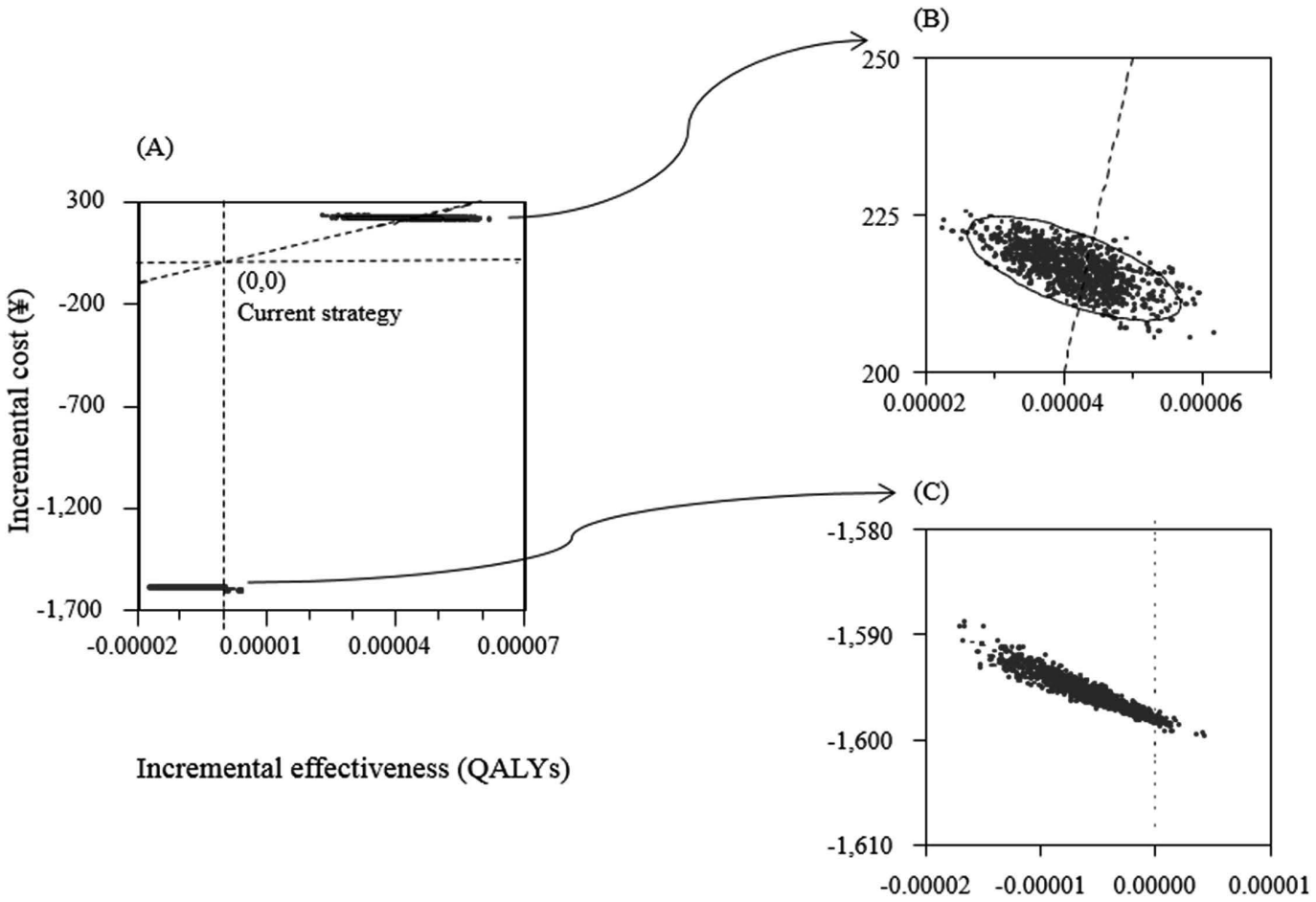

Fig 3. Results of probabilistic sensitivity analyses. (A) Scatter plot of incremental cost and incremental effectiveness per person of ≥65 PPSV–23 strategy vs. current PPSV–23 strategy and 65–80 PPSV–23 strategy vs. current PPSV–23 strategy. (B) Enlarged view of ≥65 PPSV–23 strategy vs. current PPSV–23 strategy. (C) Enlarged view of 65–80 PPSV–23 strategy vs. current PPSV–23 strategy.

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We compared 10 scenarios (with 10 PCV–13 diffusion levels) to current PPSV–23 strategy. Results showed that PCV–13 inclusion in the subsidy list has value for money (ICER = ¥378,000 or US$3,436 per QALY gained, regardless of PCV–13 diffusion level).

Since there are only a few variables which will induce the ICER to go beyond ¥1,000,000 (US$9,091) per QALY gained, we consider our results to be robust. In comparing ≥65 PPSV23 strategy with current PPSV23 strategy, the top four variables which have the biggest impact on ICER were cost per vaccine shot, IPD incidence rate, vaccine effectiveness, and percentage of vaccine serotype causing IPD. On the other hand, in comparing PCV–13 strategy with current PPSV–23, only cost per vaccine shot will change the ICER larger than ¥1,000,000 (US$9,091) per QALY.

This study has several limitations. In Japan, before the national immunisation programme was launched, some municipalities already provided subsidies to the elderly for single shot PPSV–23 from 2001 to September 2014 with a vaccine coverage of about 25% [3]. We didn't incorporate the already-vaccinated group in our model since the efficiencies of the programmes were determined by incremental difference of costs and QALYs between the

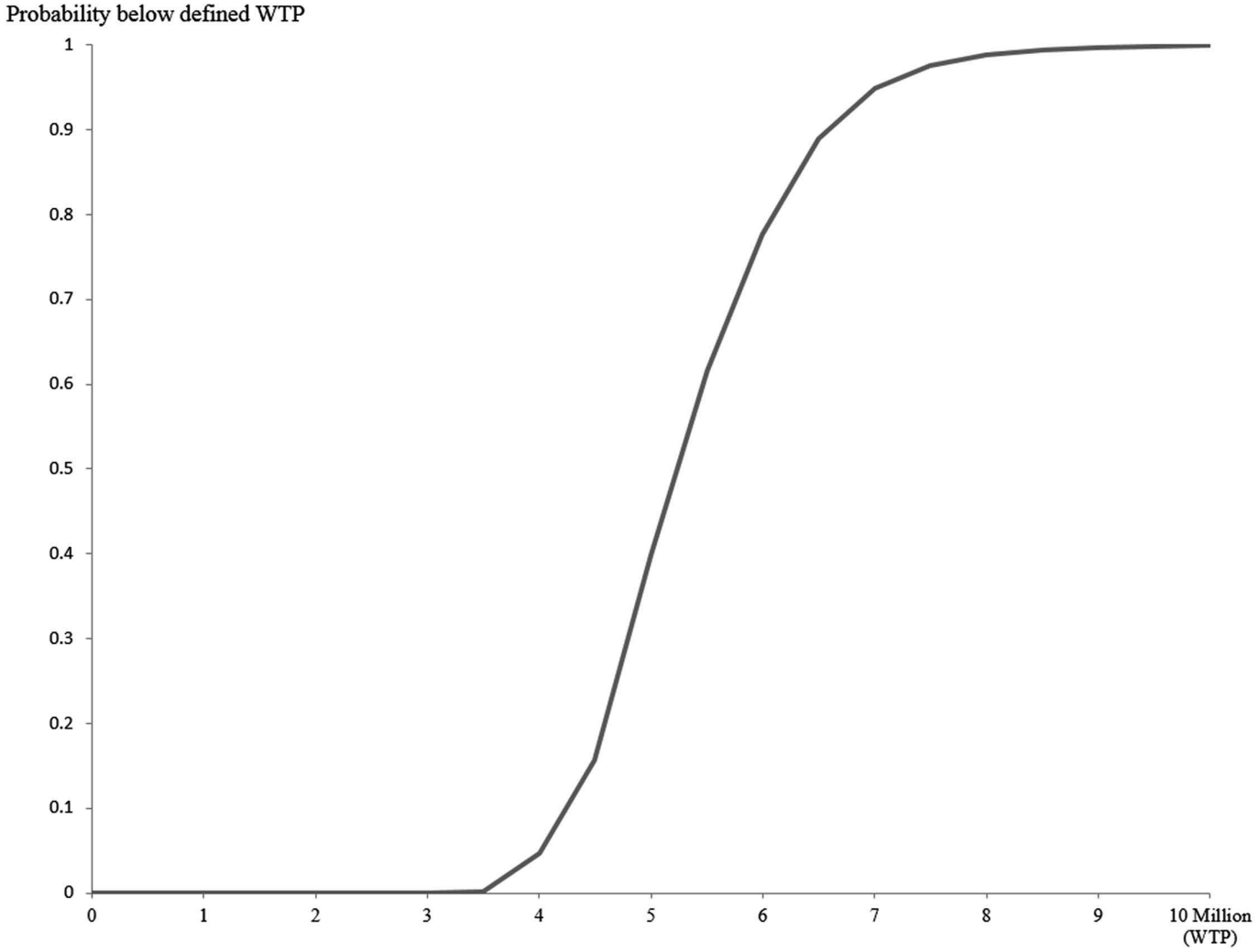

Fig 4. Cost-effectiveness acceptability curve (CEAC) of ≥65 PPSV–23 strategy vs. current PPSV–23 strategy.

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comparator and alternatives; its influence to the results should be limited. Due to insufficient data of the municipality-led PPSV–23 immunisation programmes for the elderly, we decided not incorporate this into the model. Considering that more elderly will uptake vaccine through these extra programmes, the strategy will move from current PPSV–23 strategy towards ≥ 65 PPSV–23 strategy and these results will be useful for those municipalities. We assumed that in both 65–80 PPSV–23 strategy and ≥ 65 PPSV–23 strategy, the eligible persons will uptake vaccine in the first year. Since the incidence rates of pneumococcal diseases and the vaccine effectiveness varies with age, it was difficult to predict how the results will change. Based on previous study [38], if an eligible person uptakes vaccine around 70–75 years old, it would bring more favourable results to both 65–80 PPSV–23 strategy and ≥65 PPSV–23 strategy. We didn't incorporate the herd effect of PCV–7 or PCV–13 immunisation programmes among children, which was likely to indirectly protect the elderly and thus potentially reducing the efficiencies of the immunisation programme using both vaccines. We deferred its incorporation as it might pose some bias to the result. Though several studies do provide some evidence for the existence of such an effect, further evidence is required before definite interpretations can be made. The decreasing vaccine-serotype IPD and non-invasive vaccine-type CAP cases due to serotype replacement during the 15-year cohort time were not incorporated. The replacement occurred in Japan after the launching of children's PCV immunisation programme has decreased the vaccine-serotype IPD among adults from 85% (PPSV–23) and 61% (PCV–7) in 2007 [39] to 60% (PPSV–23) and 40% (PCV–13) in 2013, respectively [19]. The advertising costs borne by manufacturers were left unaccounted. Incorporating these might bring more unfavourable results.

Several studies have compared the cost-effectiveness of the use of either PPSV–23 or PCV–13 or substitution of PPSV–23 with PCV–13 among elderly. Different studies has shown that both PPSV–23 and PCV–13 were cost-effective, and PCV–13 has high value for money than PPSV–23 [25, 40–44]. ICERs of all three PPSV–23 strategies vs. do-nothing in our study were too high to conclude that PPSV–23 immunisation programmes for the elderly were cost-effective (data in S1 Table), which was inconsistent with the results of previous studies. Inconsistencies were due to low incidence rates, low fatality rates, and low proportions of sequelae caused by *S. pneumoniae* in our study compared to those in previous studies. All 10 scenarios with different levels of share of PCV–13 have favourable ICERs but were not cost-saving compared to current strategy, which was inconsistent with the results of previous studies. The inconsistency observed was due to high vaccination cost.

Regardless of these limitations, we make efforts on literature survey to find out the available data of epidemiology and prognosis of relevant diseases which were considered to reflect the current situation of diseases caused by *S. pneumoniae* in Japan. We believe our results will provide useful results to policymakers.

Conclusion

Results of our analyses indicate switching the current strategy to ≥65 scenario or including PCV–13 into the list for single-dose subsidy to the elderly in Japan has value for money. A further budget impact analysis is awaited for well-informed policymakers.

Supporting Information

S1 Table. Cost, effectiveness and incremental cost-effectiveness ratio (vs. do-nothing) by using PPSV–23 only.
(DOCX)

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Author Contributions

Conceived and designed the experiments: SLH MK IO. Analyzed the data: SLH. Wrote the paper: SLH.

References

1. Tomczyk S, Bennett NM, Stoecker C, Gierke R, Moore MR, Whitney CG, et al. Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine among adults aged ≥65 years: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep. 2014; 63:822–5. PMID: 25233284
2. Infectious agents surveillance report (IASR) [Haienkyukin kansensyou 2013 nen genzai]. 2013;34:55–6. Japanese.
3. Naito T, Matsuda N, Tanei M, Watanabe Y, Watanabe. Relationship between public subsidies and vaccination rates with the 23-valent pneumococcal vaccine in elderly persons, including the influence of the free vaccination campaign after the Great East Japan Earthquake. J Infect Chemother. 2014; 20:450–3. doi: 10.1016/j.jiac.2014.03.004 PMID: 24767466
4. Ministry of Health, Labour and Welfare. Infectious Pneumococcal Disease (among elderly). Japanese. Available from: http://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/kenkou/kekkaku-kansenshou/haienkyukin/index_1.html
5. Squires SG, Pelletier L. Publicly-funded influenza and pneumococcal immunization programs in Canada: a progress report. Can Commun Dis Rep. 2000; 26:141–8. PMID: 11008402
6. Public Health England. Pneumococcal Polysaccharide Vaccination (PPV) Uptake. Summary report-survey year2013. Published April 2014. PHE publications gateway number: 2014042.
7. Australian Government, Department of Health and Ageing. The Australian Immunisation Handbook. 10th edition 2013.
8. Pebody RG, Leino T, Nohynek H, Hellenbrand W, Salmaso S, Ruutu P. Pneumococcal vaccination policy in Europe. Euro Surveill. 2005; 10:174–8. PMID: 16280609
9. Samson SI, Mégard Y. Overview of vaccination policies for the elderly in Western European countries. Aging Clin Exp Res. 2009; 21:210–5. PMID: 19571644
10. Ministry of Internal Affairs and communications. Popultion Estimates (October 2014). Tokyo: Statistics Bureau: 2015. Japanese.
11. Ministry of Health, Labour and Welfare. Report on Health Center Activities and Health Services for the Year 2013. Tokyo: Health and Welfare Statistics Association, 2014. Japanese.
12. Infectious Disease Sectional committee of the Tuberculosis and Infectious Disease Control Division of the MHLW. The Tenth meeting of the committee. July 16th, 2014. Japanese. Available from: http://www.mhlw.go.jp/file/05-Shingikai-10601000-Daijinkanboukouseikagakuka-Kouseikagakuka/0000058658.pdf.
13. Infectious agents surveillance report (IASR) [Seijin Haienkyuukinnsei Haien no Ekigaku]. 2014 35 (10):238–9. Japanese.
14. Ministry of Health, Labour and Welfare. The 21th life tables. Tokyo: Health and Welfare Statistics Association; 2015. Japanese.
15. Shiramoto M, Irie S, Juergens C, Yamaji M, Tamai S, Aizawa M, et al. Immunogenicity and safety of 13-valent pneumococcal conjugate vaccine when administered to healthy Japanese adults aged ≥50 years: An open-label trial. Hum Vaccin Immunother. 2014; 10:1850–8. doi: 10.4161/hv.28633 PMID: 25424792
16. Jackson LA, Gurtman A, Rice K, Pauksens K, Greenberg RN, Jones TR, et al. Immunogenicity and safety of a 13-valent pneumococcal conjugate vaccine in adults 70 years of age and older previously

vaccinated with 23-valent pneumococcal polysaccharide vaccine. Vaccine. 2013; 31:3585–93. doi: 10.1016/j.vaccine.2013.05.010 PMID: 23688527

17. Jackson LA, Gurtman A, van Cleeff M, Frenck RW, Treanor J, Jansen KU, et al. Influence of initial vaccination with 13-valent pneumococcal conjugate vaccine or 23-valent pneumococcal polysaccharide vaccine on anti-pneumococcal responses following subsequent pneumococcal vaccination in adults 50 years and older. Vaccine. 2013; 31:3594–602. doi: 10.1016/j.vaccine.2013.04.084 PMID: 23688525
18. Briggs A, Sculpher M. An introduction to Markov modelling for economic evaluation. Pharmacoeconomics. 1998; 13:397–409. PMID: 10178664
19. Infectious agents surveillance report (IASR) [Shinsyusei infuruenzakin haienkyukin kansensyou, 2014 nen 8 gatu genzai]. 2014 35(10):229–32. Japanese.
20. Watanabe A, Yanagihara K, Matsumoto T, Kohno S, Aoki N, Oguri T, et al. Nationwide surveillance of bacterial respiratory pathogens conducted by the Surveillance Committee of Japanese Society of Chemotherapy, Japanese Association for Infectious Diseases, and Japanese Society for Clinical Microbiology in 2009: general view of the pathogens' antibacterial susceptibility. J Infect Chemother. 2012; 18:609–20. doi: 10.1007/s10156-012-0434-3 PMID: 22766652
21. Takaki M, Nakama T, Ishida M, Morimoto H, Nagasaki Y, Shiramizu R, et al. High incidence of community-acquired pneumonia among rapidly aging population in Japan: a prospective hospital-based surveillance. Jpn J Infect Dis. 2014; 67:269–75. PMID: 25056072
22. Ubukata k. Juusyougata no rensa kyuujinn·haiennkyuukin kannsennsyou ni taisuru sa-berannsu no koutiku to byouinn kaiseki, sono sinndann tiryou ni kannsuru kennkyuu. Report of Health and Labour Sciences Research Grants. Tokyo: Ministry of Health, Labour, and Welfare; 2013;1–33. Japanese.
23. Ishida T, Hashimoto T, Arita M, Tojo Y, Tachibana H, Jinnai M. A 3-year prospective study of a urinary antigen-detection test for Streptococcus pneumoniae in community-acquired pneumonia: utility and clinical impact on the reported etiology. J Infect Chemother. 2004; 10:359–63. PMID: 15614462
24. Ministry of Health, Labour and Welfare. Vital statistics of Japan 2013. Tokyo: Health and Welfare Statistics Association; 2014. Japanese.
25. Smith KJ, Wateska AR, Nowalk MP, Raymund M, Nuorti JP, Zimmerman RK. Cost-effectiveness of adult vaccination strategies using pneumococcal conjugate vaccine compared with pneumococcal polysaccharide vaccine. JAMA 2012; 307,804–12. doi: 10.1001/jama.2012.169 PMID: 22357831
26. Medical Economics Division, Health Insurance Bureau, 2013. Iryou kyuhu jitai tyousa, 2013. Tokyo: Ministry of Health, Labour and Welfare 2013. Japanese.
27. The National Council on Social Security System. Simulation on costs of healthcare and long-term care. Prime minister of Japan and His Cabinet. Oct 23, 2008. Japanese.
28. National Health Insurance drug price scheme, Japan. 2014. Japanese.
29. Letter from Pfizer Inc. Japan to Ministry of Health, Labour and Welfare: about the price of Prevnar13®. June 24, 2014. Japanese.
30. Moberley S, Holden J, Tatham DP, Andrews RM. Vaccines for preventing pneumococcal infection in adults. Cochrane Database of Systematic Reviews 2013, Issue 1: . Art. No.: CD000422. doi: 10.1002/14651858.CD000422.pub3
31. Bonten MJ, Huijts SM, Bolkenbaas M, Webber C, Patterson S, Gault S, et al. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. N Engl J Med. 2015; 372:1114–25. doi: 10.1056/NEJMoa1408544 PMID: 25785969
32. Drummond MF, Sculpher MJ, Torrance GW, O'Brien BJ, Stoddart GL. Methods for economic evaluation of health care programmes. 3rd ed. Oxford: Oxford University Press; 2004.
33. Johnson D. The triangular distribution as a proxy for the beta distribution in risk analysis. The Statistician. 1997; 46:387–98.
34. Stein WE, Keblis MF. A new method to simulate the triangular distribution. Mathematical and Computer Modelling. 2009; 49:1143–7.
35. Borek A, Parlikad AK, Woodall P, Tomasella M. A risk based model for quantifying the impact of information quality. Computers in Industry. 2014; 65:354–66.
36. World Health Organization. WHO guide for standardization of economic evaluations of immunization programmes. WHO Document Production Services: Geneva, Switzerland; 2008.
37. Shiroiwa T, Sung YK, Fukuda T, Lang HC, Bae SC, Tsutani K. International survey on willingness-to-pay (WTP) for one additional QALY gained: what is the threshold of cost effectiveness? Health Econ. 2010; 19:422–37. doi: 10.1002/hec.1481 PMID: 19382128
38. Hoshi SL, Kondo M, Okubo I. Cost-effective option in launching a community-based pneumonococcal vaccination program among the elderly in Japan. Aging Research 2012; 4:e8.

39. Chiba N, Morozumi M, Sunaoshi K, Takahashi S, Takano M, Komori T, et al. Serotype and antibiotic resistance of isolates from patients with invasive pneumococcal disease in Japan. Epidemiol Infect. 2010; 138:61–8. doi: 10.1017/S0950268809990239 PMID: 19538821

40. Ogilvie I, Khoury AE, Cui Y, Dasbach E, Grabenstein JD, Goetghebeur M. Cost-effectiveness of pneumococcal polysaccharide vaccination in adults: a systematic review of conclusions and assumptions. Vaccine. 2009; 27:4891–904. doi: 10.1016/j.vaccine.2009.05.061 PMID: 19520205

41. Smith KJ, Wateska AR, Nowalk MP, Raymund M, Lee BY, Zimmerman RK. Modeling of cost effectiveness of pneumococcal conjugate vaccination strategies in U.S. older adults. Am J Prev Med. 2013; 44:373–81. doi: 10.1016/j.amepre.2012.11.035 PMID: 23498103

42. Kuhlmann A, Theidel U, Pletz MW, von der Schulenburg JM. Potential cost-effectiveness and benefit-cost ratios of adult pneumococcal vaccination in Germany. Health Econ Rev. 2012; 2:4. doi: 10.1186/2191-1991-2-4 PMID: 22828176

43. Rozenbaum MH, Hak E, van der Werf TS, Postma MJ. Results of a cohort model analysis of the cost-effectiveness of routine immunization with 13-valent pneumococcal conjugate vaccine of those aged > or = 65 years in the Netherlands. Clin Ther. 2010; 32:1517–32. doi: 10.1016/j.clinthera.2010.06.016 PMID: 20728764

44. Weycker D, Sato R, Strutton D, Edelsberg J, Atwood M, Jackson LA. Public health and economic impact of 13-valent pneumococcal conjugate vaccine in US adults aged ≥50 years. Vaccine. 2012; 30:5437–44. doi: 10.1016/j.vaccine.2012.05.076 PMID: 22728289

RESEARCH PAPER

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Association between monovalent influenza A (H1N1) pdm09 vaccine and pneumonia among the elderly in the 2009–2010 season in Japan: A case-control study

Kyoko Kondo[1,*], Kanzo Suzuki[2], Masakazu Washio[3], Satoko Ohfuji[1], Wakaba Fukushima[1], Akiko Maeda[1], Yoshio Hirota[1], and the Pneumonia in Elderly People Study Group[#]

[1]Department of Public Health; Faculty of Medicine; Osaka City University; Osaka, Japan; [2]Nagoya City University; School of Nursing; Nagoya, Japan; [3]Department of Community Health and Clinical Epidemiology; St. Mary's College; Kurume, Japan

[#]Please see Note for list of authors.

Keywords: effectiveness, elderly people, matched case-control study, monovalent influenza A (H1N1) pdm09 vaccine, pneumonia

Abbreviations: H1N1pdm, monovalent influenza A (H1N1) pdm09; TIV, trivalent seasonal influenza vaccine; COPD, chronic obstructive pulmonary disease; ADL, activities of daily living; OR, odds ratio; CI, confidence interval.

We investigated the association between monovalent influenza A (H1N1) pdm09 (H1N1pdm) vaccine and pneumonia in elderly people. Study design was a hospital-based, matched case-control study. Cases comprised patients ≥65 years old who had been newly diagnosed with pneumonia. For each case, 2 controls were defined as individuals with other diseases (not pneumonia) who were matched by sex, age, entry date, and the visited hospital. Study period was the interval from 1 September 2009 until 30 September 2010. Because a pandemic of influenza A (H1N1) occurred during study period, we analyzed selected subjects who had enrolled during the influenza A (H1N1) pandemic. We calculated the odds ratios (ORs) and 95% confidence intervals (CIs) for pneumonia in H1N1pdm-vaccinated subjects compared with unvaccinated subjects using a conditional logistic regression model to assess the association between H1N1pdm vaccine and pneumonia. The subjects during the period of the influenza A (H1N1) pandemic were 20 cases and 40 controls. Subjects who had received H1N1pdm vaccine showed a significantly decreased OR for pneumonia (OR = 0.10, 95% CI = 0.01–0.98) compared with unvaccinated subjects. In conclusion, H1N1pdm vaccination may have prevented pneumonia among the elderly during the 2009–2010 influenza A (H1N1) pandemic in Japan.

Introduction

Pneumonia is the third largest cause of death in Japan. The death rate increases with age group, and particularly high rate (more than 1,100 per a population of 100,000 people in 2007) are observed among individuals ≥80 y old.[1] With Japanese society aging at a rate not seen anywhere else in the world, prevention of pneumonia is becoming a major challenge in this country. Many studies have reported preventive relationships between influenza vaccination and hospitalization due to pneumonia or influenza among elderly people.[2-7] On the other hand, Jackson et al. reported that influenza vaccination was not associated with a reduced risk of community-acquired pneumonia after adjusting for the presence and severity of comorbidities.[8] Variations in the results of different studies reflect several confounding variables, definitions of influenza seasons, and mismatches between vaccine strains and those circulating in the community.

Few studies in Japan have examined the association between influenza vaccine and pneumonia among the elderly. Therefore we conducted a hospital-based, matched case-control study between September 2009 and September 2010 to elucidate the effectiveness of influenza vaccination in preventing pneumonia among the elderly. Due to a pandemic of influenza A (H1N1) that occurred in Japan during our study period,[9] a monovalent influenza A (H1N1) pdm09 (H1N1pdm) vaccination program was initiated in the last 10 d of October 2009. Although a

*Correspondence to: Kyoko Kondo; Email: kyou@med.osaka-cu.ac.jp

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Table 1. Characteristics of cases and controls during the 2009 influenza A(H1N1) pandemic

Characteristics*	Cases (N = 20)	Controls (N = 40)	P value
Age (mean years, range)	79.8 (65 – 95)	79.4 (65 – 97)	0.832†
Male	11 (55)	22 (55)	1.000‡
H1N1pdm vaccinated	4 (20)	14 (35)	0.232‡
Pneumococcal vaccine vaccinated	7 (35)	9 (22)	0.302‡
Underlying respiratory system disease	11 (55)	15 (37)	0.197‡
Underlying disease			
Hypertension	10 (50)	21 (53)	0.855‡
Hypercholesterolemia	2 (10)	3 (8)	1.000§
Heart disease	6 (30)	13 (33)	0.844‡
Cerebral hemorrhage, Cerebral infarction, Stroke	3 (15)	5 (13)	1.000§
Diabetes mellitus	2 (10)	8 (20)	0.471§
Kidney disease	0 (0)	2 (5)	0.548§
Smoking (past or current)	8 (40)	18 (45)	0.713‡
ADL			
Self-support	10 (50)	29 (73)	0.085‡
Others (semi-self-support, semi-bedridden, or bedridden)	10 (50)	11 (27)	

ADL: activities of daily living, H1N1pdm: monovalent influenza A (H1N1) pdm09.
*Variables are expressed as number (percent), unless otherwise specified.
† Wilcoxon rank-sum test, ‡ Chi-square test, § Fisher exact test.

trivalent seasonal influenza vaccine (TIV) vaccination program was initiated in October 2009, a seasonal epidemic did not occur.[9] Hence we investigate here the association between H1N1pdm vaccine and pneumonia in elderly people.

Results

During the influenza A (H1N1) pandemic, subjects totaled 20 cases and 40 controls from 7 medical institutions in Aichi, Kyoto, and Fukuoka.

Table 1 shows a comparison of characteristics of cases and controls. The proportion of H1N1pdm vaccination (in the preceding 6 months), pneumococcal vaccination (in the preceding 5 years), underlying respiratory system disease, hypertension, hypercholesterolemia, heart disease, cerebral hemorrhage, cerebral infarction, stroke, diabetes mellitus, kidney disease, smoking, and ADL status were not significantly different between cases and controls. In our study population, 94% of the subjects who received H1N1pdm vaccine also received TIV (17/18 subjects).

Table 2 shows the association between H1N1pdm vaccination and pneumonia among the elderly during the influenza A (H1N1) pandemic itself. Subjects who received H1N1pdm vaccine showed a significantly decreased adjusted OR for pneumonia (0.10, 95% CI = 0.01–0.98) compared with unvaccinated subjects. Pneumococcal vaccination and underlying respiratory system disease were not associated with pneumonia. The odds ratio for pneumonia increased significantly among subjects with low ADL status.

Table 2. Association between H1N1pdm vaccine and pneumonia among the elderly during the 2009 influenza A(H1N1) pandemic†

	Cases n (%)	Controls n (%)	Crude OR	95%CI	P	Adjusted OR‡	95%CI	P
H1N1pdm vaccine								
Unvaccinated	16 (80)	26 (65)	1			1		
Vaccinated	4 (20)	14 (35)	0.37	0.09–1.54	0.171	0.10	0.01–0.98	0.048
Pneumococcal vaccine								
Unvaccinated	13 (65)	31 (78)	1			1		
Vaccinated	7 (35)	9 (22)	2.35	0.55–10.0	0.249	3.46	0.50–24.1	0.209
Underlying respiratory system disease								
No	9 (45)	25 (63)	1			1		
Yes	11 (55)	15 (37)	1.80	0.66–4.89	0.248	3.65	0.76–17.4	0.105
ADL								
Self support	10 (50)	29 (73)	1			1		
Others (semi-self-support, semi-bedridden, or bedridden)	10 (50)	11 (27)	10.0	1.17–85.6	0.036	23.8	1.91–296	0.014

†The influenza A (H1N1) pandemic was defined as the weeks during which there were ≥10 reports of influenza cases reported by the sentinels in the prefectures covered by the study (see main text).
‡Model included H1N1pdm vaccination, pneumococcal vaccination, underlying respiratory system disease, and ADL.
H1N1pdm: monovalent influenza A (H1N1) pdm09, OR: odds ratio, CI: confidence interval, ADL: activities of daily living.

Discussion

In this study, the OR for pneumonia in subjects with H1N1pdm vaccination decreased significantly among elderly people during the period of the influenza A (H1N1) pandemic. Some researchers have reported that influenza vaccination reduces hospitalization due to pneumonia or influenza among elderly people living in the community.[3-6] In the 2009–2010 season, both vaccination against H1N1pdm and seasonal vaccination[2] and vaccination against MF59- adjuvant H1N1pdm[7] showed the preventive effect of influenza and pneumonia in elderly persons. Our decreased odds ratio for pneumonia suggests that during the period of the influenza A (H1N1) pandemic, H1N1pdm vaccination was associated with prevention of influenza A (H1N1) and reduction of the incidence of secondary pneumonia accompanying influenza. On the one hand, our results did not demonstrate efficacy for pneumococcal vaccination. Specifically, pneumococcal pneumonia was diagnosed in only 2 of the 20 pneumonia cases that we observed during the period of the influenza A (H1N1) pandemic, suggesting that our cases consisted predominantly of other (non-pneumococcal) pneumonias.

The presence of confounding factors is a difficult problem in studies of influenza vaccine effectiveness in the elderly.[10] Old age, underlying respiratory system disease, hypertension, hypercholesterolemia, heart disease, cerebral hemorrhage, cerebral infarction, stroke, diabetes mellitus, kidney disease, smoking, and low ADL status are associated with an increased risk of hospitalization due to pneumonia or influenza.[11] On the other hand, the vaccination rate typically is higher in healthy elderly than in weak elderly. Pneumococcus is cited as the major pathogenic bacterium in community-acquired pneumonia in the Japanese,[12] and the 23-valent pneumococcal polysaccharide vaccine has reduced the prevalence of pneumococcal pneumonia.[13] We matched controls with case patients by sex, age, entry date, and hospital, and investigated underlying respiratory system disease, hypertension, hypercholesterolemia, heart disease, cerebral hemorrhage, cerebral infarction, stroke, diabetes mellitus, kidney disease, smoking, ADL status, and pneumococcal vaccination status. The proportions of these variables were not significantly different between cases and controls. So, in multivariate model, we included underlying respiratory system disease, ADL status, and pneumococcal vaccination status that were important pathophysiological variables whether statistically significant or insignificant. Furthermore, because we did not detect an association between pneumococcal vaccination status and pneumonia, we calculated the OR adjusted for underlying respiratory disease and ADL status. The directionality of the result did not change (data not shown). We note, however, that even with adjustment for confounders, a selection bias still might have been present in the evaluation of the effectiveness of the influenza vaccine.[14]

We obtained information about vaccination status from each patient's questionnaire, but we were not able to confirm the validity of this information; this point represents a weakness of this study. Cases are expected to claim lack of vaccination more frequently than controls would, a pattern that would represent a possible information bias in our study. However, H1N1pdm vaccine non-inoculation was reported by 80% of cases and 65% of controls; pneumococcal vaccine non-inoculation was reported by 65% of cases and 78% of controls. Thus, self-reported H1N1pdm vaccine non-inoculation frequency was higher in cases than in controls, but the reverse was seen for pneumococcal vaccine non-inoculation. Therefore, information bias is considered unlikely in the context of our study.

In our study population, TIV inoculation was reported in 94% (17/18 subjects) of H1N1pdm vaccine inoculators; TIV non-inoculation was reported in 88% (37/42 subjects) of H1N1pdm vaccine non-inoculators. In other words, we considered that it was inappropriate to include TIV vaccination as an adjustment factor because of the near perfect correlation between TIV vaccination and H1N1pdm vaccination.

A smaller immune response was observed in subjects who had received the 2009–2010 seasonal influenza vaccine prior to H1N1pdm vaccination.[15] Because 94% of our study subjects were inoculated with both vaccines, we could not evaluate the effect of this factor. However, even if antibody production was reduced in response to the H1N1pdm vaccination, vaccination efficacy would have been underestimated, and so this factor would not have affected the validity of our study.

We showed significantly increased OR for pneumonia even when we adjusted for vaccination and the presence of an underlying respiratory disease in subjects with low ADL status. Fever occurred more frequently in those requiring higher care levels, and the main cause of such fevers was pneumonia.[16] Our study suggested that ADL levels would have been associated with pneumonia in the elderly.

One of the weaknesses of our study is that our matched entry date might lead to a bias. In our protocol, we enrolled controls as soon as a possible (within about 2 months) after the respective case had been enrolled. This difference in entry date between cases and controls might have given the controls more time to become vaccinated. However, controls were enrolled (on average) 10 d later than the respective case's entry; in only one instance was the control enrolled about a full 2 months after the case's entry. Therefore we do not expect that the entry dates lead to a bias.

We managed to increase the statistical power of our study by providing 2 controls for each pneumonia patient. Nevertheless, the greatest limitation of the present study was that the number of subjects was small. In practice, our 95% confidence interval was 0.01–0.98. Therefore, it would be inappropriate to calculate vaccine efficacy from our point estimate level after adjustment. However, we think that it was noteworthy that significant association was detected between H1N1pdm vaccination status and pneumonia despite the small size of our study. We expect that our study will provide a valuable data source, because our study period spanned the season in which the influenza A (H1N1) pandemic occurred. We are engaged in ongoing research to investigate the effectiveness of a seasonal influenza vaccine against pneumonia among the elderly.

Materials and Methods

Study design

We performed a hospital-based, matched case-control study in 7 hospitals (in the prefectures of Aichi, Kyoto, and Fukuoka) between September 2009 and September 2010. All subjects provided informed consent after the nature of the study had been explained. The study protocol was approved by the ethics committee at the Osaka City University Graduate School of Medicine and was performed in accordance with the Declaration of Helsinki.

Definition of cases and controls

Cases comprised patients ≥65 y old who had been newly diagnosed with pneumonia by a doctor at one of the 7 medical institutions cooperating with the study. A pneumonia diagnosis was based on clinical symptoms (cough, sputum, or fever), increased white blood cell counts or serum C-reactive protein level, and the appearance of an infiltrate on a chest radiograph at the hospitals or the clinics of the study investigators.[17]

For each case, 2 controls were selected from individuals with other diseases (not pneumonia) who were matched by sex, age (in 5-year age groups), entry date (soon after a given case's entry, within about 2 months), and the visited hospital.

Exclusion criteria were aspiration pneumonia, malignant tumor, ongoing treatment with oral corticosteroids or immunosuppressant drugs, and previous splenectomy.

Data collection

The physicians of each case or control completed a structured questionnaire regarding the following clinical information: (a) sex, age, presence of underlying respiratory system disease (pulmonary emphysema, chronic bronchitis, other chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, bronchial asthma, pulmonary tuberculosis sequelae, etc.); and (b) information relating to pneumonia (for cases): date of definite diagnosis, and test results relating to cause of pneumonia (rapid diagnosis test of influenza, detection of urinary pneumococcal antigen, Gram staining of sputum, sputum or blood culture).

Each case or control completed a self-administered questionnaire regarding the following information: presence of underlying respiratory system disease, hypertension, hypercholesterolemia, heart disease, cerebral hemorrhage, cerebral infarction, stroke, diabetes mellitus, kidney disease, smoking (never, past, or current), activities of daily living (ADL: bedridden, semi-bedridden, semi-self-support, self-support), pneumococcal vaccination (in the last 5 years), TIV vaccination (in the last 6 months), and H1N1pdm vaccination (in the last 6 months).

The H1N1pdm strain was A/California/7/2009. The 2009–2010 TIV strains were A/Brisbane/ 59/2007 (H1N1), A/Uruguay/716/2007 (H3N2), and B/Brisbane/60/2008.

Period of survey and influenza A (H1N1) pandemic

This study was initiated on 1 September 2009. We defined study period as the interval from initiation until 30 September 2010, because the vaccination program for the 2010–2011 season started on 1 October 2010.During the study period, a pandemic of influenza A (H1N1) occurred. Thus we analyzed selected subjects who had enrolled during the influenza A (H1N1) pandemic. The influenza A (H1N1) pandemic was defined as those weeks during which ≥10 influenza cases were reported by the sentinels in the prefectures covered by the study, based on data from the Infectious Disease Weekly Report and the Infectious Agents Surveillance Report. The periods meeting this definition were as follows: the period from the 38th week of 2009 until the 5th week of 2010 (between 14 September 2009 and 7 February 2010) in Aichi; the period from the 36th week of 2009 until the 3rd week of 2010

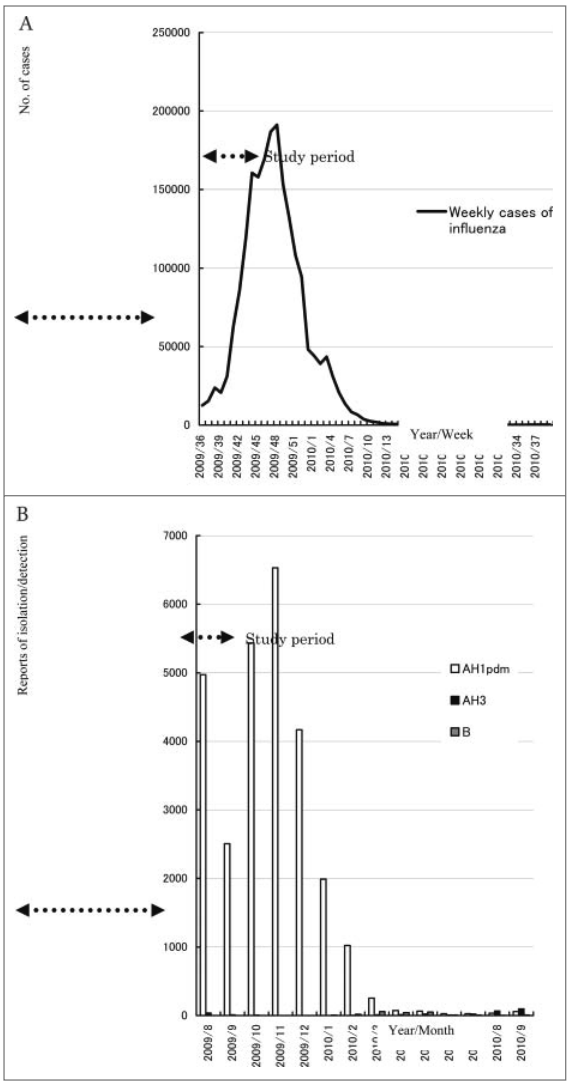

Figure 1. (**A**) Study period and weekly cases of influenza in Japan from week 35 of 2009 to week 39 of 2010. (**B**) Study period and monthly reports of isolation/detection of influenza viruses in Japan from August 2009 to September 2010.

(between 31 August 2009 and 24 January 2010) in Kyoto; and the period from the 35th week of 2009 until the 6th week of 2010 (between 24 August 2009 and 14 February 2010) in Fukuoka.[9] **Figure 1A** provides the study period and plotting the numbers of weekly cases of influenza in Japan from week 35 of 2009 to week 39 of 2010. **Figure 1B** provides the study period and the numbers of monthly reports of isolation/detection of influenza viruses in Japan from August 2009 to September 2010.[9] A seasonal epidemic did not occur in the prefectures covered by the study.

Statistical analysis

Characteristics of cases and controls were compared using a Wilcoxon rank-sum test and χ^2 test, as appropriate.

We calculated the odds ratios (ORs) and 95% confidence intervals (CIs) for pneumonia in H1N1pdm-vaccinated subjects compared with those in unvaccinated subjects using a conditional logistic regression model.

We adjusted for pneumococcal vaccination (yes in the last 5 years, no), underlying respiratory system disease (yes, no), and ADL (other (bedridden, semi-bedridden, or semi-self-support), self-support) in multivariate analyses.

The significance level for statistical analysis was set at $P < 0.05$. Analyses were performed using SAS version 9.3 software (SAS Institute, Cary, NC, USA).

Conclusions

We conducted a hospital-based, matched case-control study between September 2009 and September 2010 to elucidate the association between influenza vaccine and pneumonia in elderly people. Our results indicate that H1N1pdm vaccination may have prevented pneumonia among the elderly during the 2009–2010 influenza A (H1N1) pandemic in Japan

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Authors' Contributions

KK designed the study, did the statistical analysis, interpreted the results and wrote the first draft of the article. KS and MW designed the study, collected data, interpreted the results, and edited drafts of the article. SO, WF, AM and YH designed the study, interpreted the results and edited drafts of the article. Members of the Pneumonia in Elderly People Study Group (Note) collected data and interpreted the results. All authors read and approved the final draft of the article.

Note

The other members of the Pneumonia in Elderly People Study Group are listed with their affiliation: Noriko Kojimahara (Tokyo Women's Medical University); Sakae Kan, Toshinobu Yamamoto (Kaisei Hospital); Chiharu Ohta (Asahi Rousai Hospital); Kazuhide Yamamoto (Kazu Clinic); Yoichi Nakanishi (Graduate School of Medical Sciences, Kyushu University); Nobumitsu Fujisawa, Hideki Tashiro, Yoshihisa Shimizu, Noriko Fujimoto, Yasuomi Yoneshima, Kenji Fukuda (St. Mary's Hospital); Masahiko Taketomi (Doukai Clinic); Tomoaki Iwanaga, Hiroko Nogami (Fukuoka National Hospital); Koichi Takano (Nishifukuoka Hospital); Ken Tonegawa, Yoshimitsu Hayashi (Nagoya City Koseiin Geriatric Hospital); Seiichiro Imai (Graduate School of Medicine, Kyoto University); Ikuo Ikeda (Ikeda Clinic).

References

1. Ministry of Health, Labour and Welfare. Annual Statistical Report of National Health Conditions and Health Promotion 2009. Tokyo: Health, Labour and Welfare Statistical Association, 2009. [in Japanese].
2. Chan TC, Hung IF, Luk JK, Shea YF, Chan FH, Woo PC, Chu LW. Efficacy of dual vaccination of pandemic H1N1 2009 influenza and seasonal influenza on institutionalized elderly: a one-year prospective cohort study. Vaccine 2011; 29:7773–8; PMID:21821084; http://dx.doi.org/10.1016/j.vaccine.2011.07.112
3. Mullooly JP, Bennett MD, Hornbrook MC, Barker WH, Williams WW, Patriarca PA, Rhodes PH. Influenza vaccination programs for elderly persons: cost-effectiveness in a health maintenance organization. Ann Intern Med 1994; 121:947–52; PMID:7978721; http://dx.doi.org/10.7326/0003-4819-121-12-199412150-00008
4. Nichol KL, Nordin JD, Nelson DB, Mullooly JP, Hak E. Effectiveness of influenza vaccine in the community-dwelling elderly. N Engl J Med 2007; 357:1373–81; PMID:17914038; http://dx.doi.org/10.1056/NEJMoa070844
5. Nichol KL, Wuorenma J, Sternberg T. Benefits of influenza vaccination for low-, intermediate-, and high-risk senior citizens. Arch Intern Med 1998; 158:1769–76; PMID:9738606; http://dx.doi.org/10.1001/archinte.158.16.1769
6. Baxter R, Ray GT, Fireman BH. Effect of influenza vaccination on hospitalizations in persons aged 50 years and older. Vaccine 2010; 28:7267–72; PMID:20832494; http://dx.doi.org/10.1016/j.vaccine.2010.08.088
7. Gefenaite G, Tacken M, Bos J, Stirbu-Wagner I, Korevaar JC, Stolk RP, Wolters B, Bijl M, Postma MJ, Wilschut J, et al. Effectiveness of A(H1N1)pdm09 influenza vaccine in adults recommended for annual influenza vaccination. PLoS One 2013; 8:e66125; PMID:23840413; http://dx.doi.org/10.1371/journal.pone.0066125
8. Jackson ML, Nelson JC, Weiss NS, Neuzil KM, Barlow W, Jackson LA. Influenza vaccination and risk of community-acquired pneumonia in immunocompetent elderly people: a population-based, nested case-control study. Lancet 2008; 372:398–405; PMID:18675690; http://dx.doi.org/10.1016/S0140-6736(08)61160-5
9. IASR. (2010); 2009/10 influenza season, Japan. Infectious Agents Surveillance Report 2010, 31:248–250.
10. Jackson LA, Nelson JC, Benson P, Neuzil KM, Reid RJ, Psaty BM, Heckbert SR, Larson EB, Weiss NS. Functional status is a confounder of the association of influenza vaccine and risk of all cause mortality in seniors. Int J Epidemiol 2006; 35:345–52; PMID:16368724; http://dx.doi.org/10.1093/ije/dyi275
11. Torres A, Peetermans WE, Viegi G, Blasi F. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. Thorax 2013; 68:1057–65; PMID:24130229; http://dx.doi.org/10.1136/thoraxjnl-2013-204282
12. Ishida T, Hashimoto T, Arita M, Ito I, Osawa M. Etiology of community-acquired pneumonia in hospitalized patients. A 3-year prospective study in Japan. Chest 1998; 114:1588–93; PMID:9872193; http://dx.doi.org/10.1378/chest.114.6.1588
13. Maruyama T, Taguchi O, Niederman MS, Morser J, Kobayashi H, Kobayashi T, D'Alessandro-Gabazza C, Nakayama S, Nishikubo K, Noguchi T, et al. Efficacy of 23-valent pneumococcal vaccine in preventing pneumonia and improving survival in nursing home residents: double blind, randomised and placebo controlled trial. BMJ 2010; 340:c1004; PMID:20211953; http://dx.doi.org/10.1136/bmj.c1004
14. Fukushima W, Hayashi Y, Mizuno Y, Suzuki K, Kase T, Ohfuji S, Fujieda M, Maeda A, Hirota Y. Selection bias in evaluating of influenza vaccine effectiveness: a

lesson from an observational study of elderly nursing home residents. Vaccine 2008; 26:6466–9; PMID:18582520; http://dx.doi.org/10.1016/j.vaccine.2008.06.038

15. Ohfuji S, Fukushima W, Deguchi M, Kawabata K, Yoshida H, Hatayama H, Maeda A, Hirota Y. Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine among pregnant women: lowered antibody response by prior seasonal vaccination. J Infect Dis 2011; 203:1301–8; PMID:21459817; http://dx.doi.org/10.1093/infdis/jir026
16. Yokobayashi K, Matsushima M, Watanabe T, Fujinuma Y, Tazuma S. Prospective cohort study of fever incidence and risk in elderly persons living at home. BMJ Open 2014; 4:e004998; PMID:25009132; http://dx.doi.org/10.1136/bmjopen-2014-004998
17 Kawakami K, Ohkusa Y, Kuroki R, Tanaka T, Koyama K, Harada Y, Iwanaga K, Yamaryo T, Oishi K. Effectiveness of pneumococcal polysaccharide vaccine against pneumonia and cost analysis for the elderly who receive seasonal influenza vaccine in Japan. Vaccine 2010; 28:7063–9; PMID:20723631; http://dx.doi.org/10.1016/j.vaccine.2010.08.010

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Corrigendum

Article title: "Association between monovalent influenza A (H1N1) pdm09 vaccine and pneumonia among the elderly in the 2009-2010 season in Japan: A case-control study"
Authors: Kyoko Kondo, Kanzo Suzuki, Masakazu Washio, Satoko Ohfuji, Wakaba Fukushima, Akiko Maeda, Yoshio Hirota, and the Pneumonia in Elderly People Study Group
Journal: *Human Vaccines & Immunotherapeutics*
Bibliometrics: Volume 11, Issue 05, pages 1088-1093
DOI: 10.1080/21645515.2015.1016668

An early version of Figure 1 was used in the final version of the paper. The final version of Figure 1 can be found below:

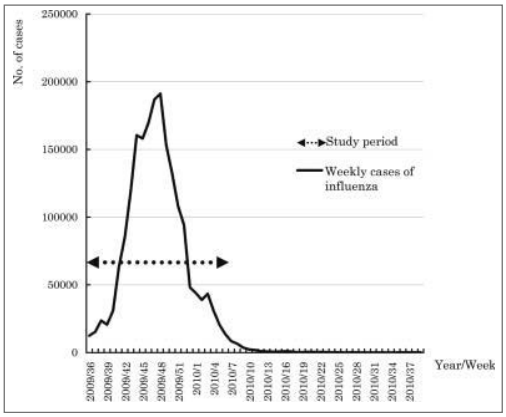

Figure 1A. Study period and weekly cases of influenza in Japan from week 35 of 2009 to week 39 of 2010.

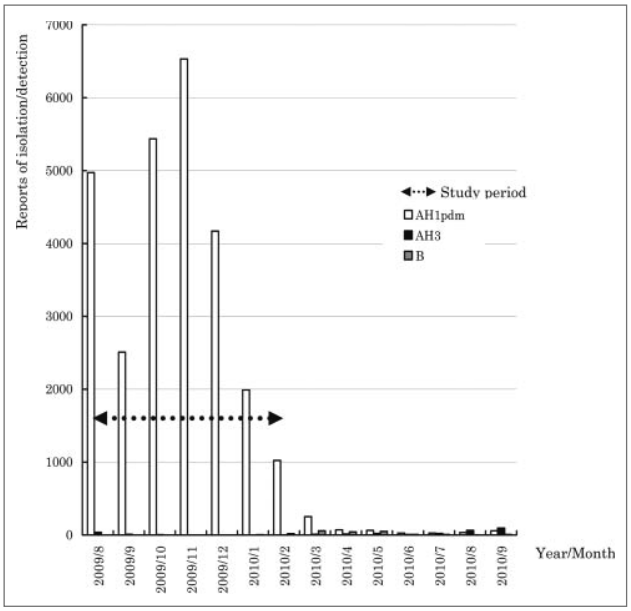

Figure 1B. Study period and monthly reports of isolation/detection of influenza viruses in Japan from August 2009 to September 2010

The authors apologize for any inconvenience caused.

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Detection of respiratory viruses in gargle specimens of healthy children

Saeko Morikawa*, Satoshi Hiroi, Tetsuo Kase

Division of Virology, Department of Infectious Diseases, Osaka Prefectural Institute of Public Health, 1-3-69, Nakamichi, Higashinari-ku, Osaka 537-0025, Japan

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ABSTRACT

Background: Respiratory tract viral infection is one of the most common and important diseases in children. Polymerase chain reaction (PCR) tests are often used to detect viruses in samples, it is difficult to interpret the clinical significance of PCR positivity, which may reflect a past, imminent or active asymptomatic infection due to their high sensitivity. Although single respiratory viruses have been detected in samples from children with symptoms, other respiratory viruses can also be detected simultaneously. However, the clinical importance of these findings for the symptoms is not known.
Objectives: To investigate the prevalence of respiratory viruses among children without any symptoms such as acute respiratory illness and/or fever.
Study design: From week twenty-five 2013 to week twenty-six 2014, gargle samples were collected from children once a week and these samples were subjected to real-time PCR to detect respiratory viruses. On each sampling day, we asked the parents about their children's health condition.
Results: Among the 286 samples collected, 200 were from asymptomatic children. In the asymptomatic condition, human parechovirus, adenovirus, enterovirus, rhinovirus, coronavirus 229E and HKU1 were observed in 45 episodes. In samples from symptomatic children, parainfluenza viruses, respiratory syncytial virus and coronavirus OC43 were detected in addition to those mentioned above.
Conclusions: Various viruses of different species were detected in the specimens from the children regardless of their health status. It might be speculated that host factors such as the function of the immune system influence the clinical outcome of the infection. However, this needs to be studied further.

1. Background

Respiratory tract viral infection is one of the most common and important disease conditions in children. Recently, PCR based assays have made it possible for novel viruses to be discovered, leading to appraisal of the clinical impacts of these viruses and several other well-known respiratory viruses [1–4]. Some of these viruses are detected alone in specimens from patients with respiratory symptoms (sometimes in those of inpatients) but their pathogenicity is not clear because they are detected simultaneously with other viruses in many cases [5–7]. As a result, the clinical importance of these findings for the symptoms is not known.

2. Objectives

In this study, we investigated how often and what respiratory viruses were detected in specimens from asymptomatic children. Gargle specimens (obtained by rinsing the throat with distilled water) were collected from children once a week and the samples were subjected to two-step real-time PCR to detect respiratory viruses. Singleplex real-time PCR procedures were employed for detection of the following 15 respiratory viral pathogens: parainfluenza viruses (PIV) 1–4, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), enterovirus (EV)/rhinovirus (RV), human bocavirus (hBoV), human parechovirus (hPeV), adenovirus (AdV), and human coronaviruses (hCoV) OC43, NL63, 229E, and HKU-1 (Table 1), and one-step real-time reverse transcription (RT)–PCR was used for detection of influenza viruses (FluV) A and B (Table 1).

Abbreviations: PCR, polymerase chain reaction; PIV, parainfluenza virus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; EV, enterovirus; RV, rhinovirus; RVA, rhinovirus genogroup A; RVB, rhinovirus genogroup B; RVC, rhinovirus genogroup C; hBoV, human bocavirus; hPeV, human parechovirus; AdV, adenovirus; hCoV, human coronavirus; FluV, influenza virus; RT, reverse transcription.

* Corresponding author. Tel.: +81 66972 1321; fax: +81 66972 2393.
E-mail addresses: morikawa@iph.pref.osaka.jp (S. Morikawa), hiroi@iph.pref.osaka.jp (S. Hiroi), kasetetsuo@iph.pref.osaka.jp (T. Kase).

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1386-6532/

Table 1
Primers and probes used in this study.

Virus	Target	Product size(bp)	Specific primers and probes	Detection limit (copy/uL)	Reference
PIV1	HN	135	Antisense 5' GTCCTTCCTGCTGGTGTGTTAAT 3'	6.55×10^2	
			Sense 5' CCAACCTACAAGGCAACAACATC 3'		[27]
			Probe 5' (FAM)CAAACGATGGCTGAAAA(TAMRA) 3'		
PIV3	HN	161	Antisense 5' TTGTTATAGTGTGTAATGCAGCTCGT 3'	5.30×10^2	
			Sense 5' GGGAGCATTGTGTCATCTGTCA 3'		[27]
			Probe 5' (FAM)CCCAGTCATAACTTACTC(TAMRA) 3'		
PIV2	NP	65	Antisense 5' TCYTCAGCTAATGCTTCRAARGC 3'	1.0×10^2	
			Sense 5' ATTCCAGATGCTCGATCAACTATG 3'		[28]
			Probe 5' (FAM)AGCACYTCTCCTCTGG(TAMRA) 3'		
PIV4	NP	123	Antisense 5' ATGTGGCCTGTAAGGAAAGCA 3'	1.0×10^1	
			Sense 5' CAAAYGATCCACAGCAAAGATTC 3'		[29]
			Probe 5' (FAM)GTATCATCATCTGCCAAATCGGCAATTAAACA(TAMRA) 3'		
RSV	F	89	Antisense 5' CGATTTTTATTGGATGCTGTACATTT 3'	2.22×10^2	
			Sense 5' AACAGATGTAAGCAGCTCCGTTATC 3'		[30]
			Probe 5' (FAM)TGCCATAGCATGACACAATGGCTCCT(TAMRA) 3'		
hPMV	M	152	Antisense 5' CATCAGCCYYATCWGTGTTTCTTAAAA 3'	2.47×10^2	
			Sense 5' GGCTCCATGCAAATATGAAGTG 3'		[31]
			Probe 5' (FAM)CTAACGAGTGTGCGCAAG(TAMRA) 3'		
EV/RV	5'NTR[b]	203	Antisense 5' GAAACACGGACACCCAAAGTAGT 3'	Echo 9.76×10	
			Sense 5' AGCCTGCGTGGCKGCC 3'	RVC 2.98×10^2	[32]
			Probe 5' (FAM) CTCCGGCCCCTGAATGYGGCTAA(TAMRA) 3'		
hBoV	NP-1	75	Antisense 5' TGGACTCCCTTTTCTTTTGTAGGA 3'	5.05×10^2	
			Sense 5' GCACAGCCACGTGACGAA 3'		[33]
			Probe 5' (FAM)TGAGCTCAGGGAATATGAAAGACAAGCATCG(TAMRA) 3'		
hCoV229E	NC	80	Antisense 5' TCTTTTCCACCGTGGCTTTT 3'	1.0×10^2	
			Sense 5' CTGCCAAGAGTCTTGCTCGTT 3'		[28]
			Probe 5' (FAM)AGAACAAAAGCATGAAATG(TAMRA) 3'		
hCoVNL63	NC	61	Antisense 5' CGAGGACCAAAGCACTGAATAA 3'	1.17×10^2	
			Sense 5' AACCTCGTTGGAAGCGTGTT 3'		[28]
			Probe 5' (FAM)ATTTTCCTCTCTGGTAG(TAMRA) 3'		
hCoVOC43	NC	67	Antisense 5' GCTGAGGTTTAGTGGCATCCTT 3'	2.19×10^2	
			Sense 5' GACATGGCTGATCAAATTGCTAGT 3'		[28]
			Probe 5' (FAM)TCTGGCAAAACTTGG(TAMRA) 3'		
hCoV HKU	ORF 1a/b	61	Antisense 5' CATTCATTCGCAAGGCGATA 3'	1.11×10^2	
			Sense 5' CCCGCAAACATGAATTTTGTT 3'		[28]
			Probe 5' (FAM)AATCTATCACCATGTGAA (TAMRA) 3'		
hPeV	5'NTR	194	Antisense 5' GGCCCCWGRTCAGATCCAYAGT 3′	1.0×10^2	
			Sense 5' GTAACASWWGCCTCTGGGSCCAAAAG 3′		[34]
			Probe 5'(FAM)CCTRYGGGTACCTYCWGGGCATCCTTC(TAMRA) 3′		
AdV(ACDF)	Hexon	85	Antisense 5' AAACTTGTTATTCAGGCTGAAGTACGT3'	1.0×10^2	
			Sense 5' CCAGGACGCCTCGGAGTA 3'		[35]
			Probe 5' (FAM)AGTTTGCCCGCGCCACCG(TAMRA) 3'		
AdV(BE)	Hexon	81	Antisense 5' CTTGTTCCCCAGACTGAAGTAGGT 3'	1.0×10^2	
			Sense 5' GGACAGGACGCTTCGGAGTA 3'		[35]
			Probe 5' (FAM)CAGTTCGCCCGYGCMACAG(TAMRA) 3'		
FluV typeA	MP	149	Antisense 5' TGACAGRATYGGTCTTGTCTTTAGCCAYTCCA	7.5[a]	
			Sense 5' CCMAGGTCGAAACGTAYGTTCTCTCTATC		[36]
			Probe 5' (FAM)ATYTCGGCTTTGAGGGGGCCTG(MGB) 3'		
FluV AH1pdm09	HA	187	Antisense 5' TGTTTCCACAATGTARGACCAT	6.8[a]	
			Sense 5' AGAAAAGAATGTAACAGTAACACACTCTGT		[36]
			Probe 5' (FAM)CAGCCAGCAATRTTRCATTTACC(MGB) 3'		
FluV AH3	HA	178	Antisense 5'GTCATTGGGRATGCTTCCATTTGG	7.1[a]	
			Sense 5' CTATTGGACAATAGTAAAACCGGGRGA		[36]
			Probe 5' (FAM)AAGTAACCCCKAGGAGCAATTAG(MGB) 3'		
FluV B	NS	105	Antisense 5'GTKTAGGCGGTCTTGACCAG	8.2[a]	
			Sense 5' GGAGCAACCAATGCCAC		[37]
			Probe 5' (FAM)ATAAACTTTGAAGCAGGAAT(MGB) 3'		

[a] From reference data.
[b] NTR: non translated region.

3. Study design

3.1. Subjects

Twelve children aged 3–10 years old were enrolled. From week twenty-five 2013 to week twenty-six 2014, throat gargle samples were obtained from the children once a week. Their parents noted the existence of respiratory symptoms (cough, sore throat or nasal mucus) and systemic symptoms (fever or rash) at the time of sampling. Written informed consent was obtained from the parents.

3.2. Molecular analysis

Nucleic acids were extracted from 200 μL specimens using the Magtration System with a MagDEA viral DNA/RNA 200 kit (Precision System Science Co., Ltd., Chiba, Japan) as 50 μL of elution volume. RT reactions were performed using a ReverTra Ace qPCR RT kit (TOYOBO Co., Ltd., Osaka, Japan) following the manufacturer's instructions. The cDNA was then amplified using Realtime PCR Master Mix (TOYOBO) with a total volume of 25 μL. Each sample was amplified containing primers and probes specific for each of the targets as described in Table 1 [27–37]. The sensitivity of each of the

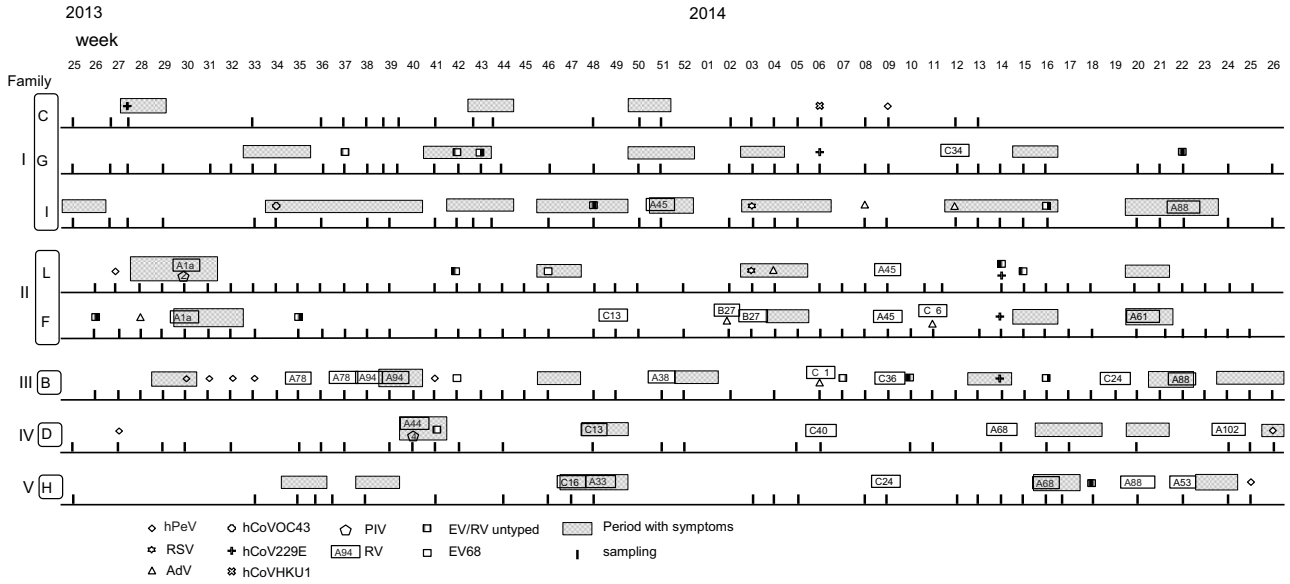

Fig. 1. Relations between viruses detected in gargle specimens of 8 children and respiratory and/or systemic symptoms. Detected viruses are shown using the symbols noted in the explanatory notes. Vertical lines indicate sampling time. The letters and numbers in rectangles indicate RV genotypes. Children C, G and I are three of four siblings. F and L are a girl and her older brother.

real-time PCR methods was evaluated by detecting serial dilutions of quantitated plasmids that contained each target DNA clone. For detection of FluV A and B, we used the one-step real-time RT–PCR method because of its increased sensitivity. Enteroviruses and rhinoviruses were genotyped by direct sequencing. Amplification of the VP4/VP2 region of the enterovirus or rhinovirus for typing was performed with semi-nested RT–PCR as previously described [8]. The purified PCR products were subjected to direct sequencing with a BigDye Terminator v1.1 kit as per the manufacturer's instructions (Applied Biosystems, CA, USA). Sequence analysis was performed using the DNADynamo program (Blue Tractor Software, UK). Using MEGA5.2 (Tamura et al., 2011, Ver5.2.2), we employed the neighbor-joining method [14] to construct phylogenetic trees from the VP4/VP2 region (420nt) sequences retrieved from GenBank of prototype isolates of each rhinovirus type commonly used in epidemiologic studies of human rhinoviruses [9–11] and new types proposed previously [9,12,13]. Genotypes were assigned on the basis of their clustering with known prototype reference strains.

4. Results

Four children were excluded because of insufficient sampling frequency. For the asymptomatic condition, the criteria were the absence of respiratory symptoms (cough, sniffle or sore throat) and systemic symptoms (fever or rash) from one week before to two days after sampling. Of the 286 samples, 200 were from children who were asymptomatic (Fig. 1). When RNA was EV/RV positive by real-time PCR but the viral VP4/VP2 region could not be amplified by semi-nested RT–PCR, we defined it as EV/RV untyped. The threshold cycle (Ct.) of real-time PCR is a relative measure of the concentration of the target in the PCR reaction. If the Ct. value of the EV/RV real-time PCR test is high (over 36.0), the nucleic acids cannot be amplified by the semi-nested PCR used for genotyping (data not shown).

Of the 200 samples, 45 (22.5%) were real-time PCR positive. Four of the 45 positive samples contained two viruses. The prevalence of respiratory viruses among asymptomatic children varied from 9.1% (1/11) to 42.9% (15/35) and that in the symptomatic period

Table 2
Prevalence of respiratory viruses in gargle specimens of children.

Family	Child	Age (years)	Sex	Total no. of sample	Condition (n)	Prevalence% (positive sample)
I	C	9	M	24	Asymptomatic (19)	10.5 (2)
					Symptomatic (5)	20.0 (1)
	G	6	F	38	Asymptomatic (27)	14.8 (4)
					Symptomatic (11)	18.2 (2)
	I	3	M	33	Asymptomatic (11)	9.1 (1)
					Symptomatic (22)	31.8 (7)
II	L	6	M	44	Asymptomatic (35)	14.3 (5)
					Symptomatic (9)	44.4 (4)
	F	3	F	45	Asymptomatic (36)	25.0 (9)
					Symptomatic (9)	22.2 (2)
III	B	4	M	48	Asymptomatic (35)	42.9 (15)
					Symptomatic (13)	30.8 (4)
IV	D	3	M	27	Asymptomatic (20)	20.0 (4)
					Symptomatic (7)	57.1 (4)
V	H	5	F	27	Asymptomatic (19)	26.3 (5)
					Symptomatic (8)	37.5 (3)

Table 3
Detection of respiratory viruses in gargle specimens of children.

Virus	Condition	No. of detections(%)
Enterovirus 68	Asymptomatic	1 (0.5)
	Symptomatic	1 (1.2)
Human rhinovirus A	Asymptomatic	10 (5.0)
	Symptomatic	10 (11.6)
Human rhinovirus B	Asymptomatic	2 (1.0)
	Symptomatic	0 (0)
Human rhinovirus C	Asymptomatic	8 (4.0)
	Symptomatic	2 (2.3)
Human parechovirus	Asymptomatic	8 (4.0)
	Symptomatic	2 (2.3)
Human coronavirus HKU-1	Asymptomatic	1 (0.5)
	Symptomatic	0 (0)
Human coronavirus 229 E	Asymptomatic	3 (1.5)
	Symptomatic	2 (2.3)
Human coronavirus OC43	Asymptomatic	0 (0)
	Symptomatic	1 (1.2)
Parainfluenza virus 2	Asymptomatic	0 (0)
	Symptomatic	1 (1.2)
Parainfluenza virus 4	Asymptomatic	0 (0)
	Symptomatic	1 (1.2)
RS virus	Asymptomatic	0 (0)
	Symptomatic	2 (2.3)
Adenovirus	Asymptomatic	5 (2.5)
	Symptomatic	2 (2.3)
EVRV untyped	Asymptomatic	11 (5.5)
	Symptomatic	5 (5.8)

ranged from 18.2% (2/11) to 57.1% (4/7) (Table 2). The most frequently detected virus was RV genogroup A (RVA) ($n = 10$) (Table 3). EV/RV from 11 samples could not be genotyped. Two of the 4 samples with codetection contained RVC and adenovirus, one RVB and adenovirus, and one EV/RV untyped and hCoV 229E.

Human PeV was detected in 8 samples. After hPeV was detected in a sample from a symptomatic child, it was subsequently detected for more than three weeks without any symptoms (Fig. 1, Child B).

In samples from symptomatic children, PIV, RSV and hCoV OC43 were detected in addition to the viruses detected in those from asymptomatic children (27/86; 31.4%). The most commonly detected virus was RVA (10/27; 37.0%). Among the 27 samples, 2 contained PIV and RVA. FluV, hBoV and hMPV were not detected.

5. Discussion

Gargle specimens from 8 children were collected once a week and the samples were subjected to real-time PCR to detect respiratory viruses. RVs and EV/RV untyped were the viruses most frequently detected in samples from asymptomatic children. Current diagnosis of respiratory infections is mainly done using PCR methods. Due to their high sensitivity, it is difficult to determine the exact explanation for positivity in individual participants (e.g., post-viral shedding, asymptomatic infection, or incubation before symptomatic infection). We were able to clarify the active asymptomatic infection by testing gargle specimens of the same children once a week for one year.

RVs are most commonly isolated from persons experiencing mild upper respiratory illness (common cold). Recent studies have reported that those viruses are responsible for severe infections of the lower respiratory tract in children. These viruses play a critical role in exacerbating asthma and chronic lung diseases [15,16]. However, most studies were conducted with symptomatic patients. Few studies have investigated the existence of the viruses in children without any respiratory symptoms [17,18]. One study reported that, after the onset of symptomatic respiratory infection, rhinovirus RNA may take a long time (5–6 weeks) to disappear from nasal mucus [19]. In this study, the children who could gargle might have been relatively older, but RVs were often detected in their throats at a time without symptoms. It seems that RV infection is in most cases asymptomatic or mild. As the sensitivity of the real-time PCR was 100 copies, it can be assumed that the virus might have replicated to some extend. The same RV genotype was detected in two consecutive samples of a child and another RV genotype was detected in the next sample. These findings suggest that RVs do not exist in the upper respiratory tract for a long time even if a child does not show symptoms which were probably the result of interferon response to a virus multiplication.

HPeV was also detected in samples from asymptomatic children. Recent studies have investigated the involvement of hPeVs in respiratory diseases, reporting a low frequency of detection and a lack of clear disease association. In addition to a low hPeV prevalence in respiratory samples, a high rate of coinfection with other respiratory viruses has been observed in hPeV-positive samples [1,20]. With monthly sampling, hPeV was detected in the stools of 48% of healthy Finnish infants by the age of 22 months [21]. In this study, the duration of parechovirus shedding in gargle specimens was calculated to be 3 weeks after the disappearance of the respiratory symptoms.

On the other hand, for PIVs, RSV, and hCoV OC43, which were detected only when clinical symptoms were seen, it is thought that, if these viruses grow in the airway, certain host reactions such as respiratory symptoms or fever will be triggered [22–26].

FluV, hBoV, hMPV and hCoV NL63 were not detected during the study period, probably because the children in this study did not live in a viral epidemic area.

Since various viruses were detected in the children regardless of their health condition, it might be speculated that the clinical outcome of the respiratory viral infection is affected predominantly such as the function of immune system. Most respiratory viruses infect the upper or lower airway and replicate in airway epithelial cells. In patients with normal immunity, these viruses are cleared immediately and it is generally thought that prolonged infection is rare. Therefore these respiratory viruses must repeat human-to-human transmission to continue to be present in the human population. As PCR is a nucleic acid amplification method, it remains unknown whether the respiratory viruses detected in the specimens from asymptomatic children are infective or not. Respiratory viral infection without any symptoms may play an important role in the viral circulation in human populations.

Competing interest

None declared.

Ethical approval

This study was approved by the Osaka Prefectural Institute of Public Health ethical committee (No. 1302-05-01).

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References

[1] M. Debiaggi, F. Canducci, E.R. Ceresola, M. Clementi, The role of infections and co-infections with newly identified and emerging respiratory viruses in children, Virol. J. 9 (2012) 247.
[2] S.A. Ali, J.E. Gern, T.V. Hertert, K.M. Edwards, M.R. Griffin, E.K. Miller, et al., Real-world comparison of two molecular methods for detection of respiratory viruses, Virol. J. 8 (2011) 332.

[3] B.G. Van den Hoogen, J.C. de jong, J. Groen, T. Kuiten, R. de Groot, R.A. Fouchier, A.D. Osterhaus, A newly discovered human pneumovirus isolated from young children with respiratory tract disease, Nat. Med. 7 (2001) 719–724.
[4] T. Allander, M.T. Tammi, M. Eriksson, A. Bjerkner, A. Tiveljung-Lindell, B. Andersson, Cloning of a human parvovirus by molecular screening of respiratory tract samples, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 12891–12896.
[5] M.M. Prill, M.K. Iwane, K.M. Edward, J.V. Williams, G.A. Weinberg, M.A. Staat, et al., Human coronavirus in young children hospitalized for acute respiratory illness and asymptomatic controls, Pediatr. Infect. Dis. J. 31 (3) (2012) 235–240.
[6] M.L. von Linstow, M. Hogh, B. Hogh, Clinical and epidemiologic characteristics of human bocavirus in Danish infants: results from a prospective birth cohort study, Pediatr. Infect. Dis. J. 27 (2008) 897–902.
[7] Y. Jin, X.H. Yuan, Z.P. Xie, H.C. Gao, J.R. Song, R.F. Zhang, et al., Prevalence and clinical characterization of a newly identified human rhinovirus C species in children with acute respiratory tract infections, J. Clin. Microviol. 47 (2009) 2895–2900.
[8] H. Ishiko, Y. Shimada, M. Yonaha, O. Hashimoto, A. Hayashi, K. Sakae, N. Takeda, Molecular diagnosis of human enteroviruses by phylogeny-based classification by use of the VP4 sequence, J. Infect. Dis. 185 (6) (2002) 744–754.
[9] C.L. McIntyre, N.J. Knowles, P. Simmonds, Proposals for the classification of human rhinovirus species A, B and C into genotypically assigned types, J. Gen. Virol. 94 (2013) 1791–1806.
[10] C. Henquell, A. Mirand, A.L. Deusebis, C. Regagnon, C. Archimbaud, M. Chambon, et al., Prospective genotyping of human rhinoviruses in children and adults during the winter of 2009–2010, J. Clin. Virol. 53 (2012) 280–284.
[11] A. Kaida, H. Kubo, K. Takakura, M. Togawa, M. Shiomi, U. Koudera, N. Iritani, Molecular epidemiology of human rhinovirus C in patients with acute respiratory tract infections in Osaka City, Japan, Jpn. J. Infect. Dis. 64 (2011) 488–492.
[12] P. Simmonds, C. McIntyre, C. Savolainen-kopra, C. Tapparel, I.M. Mackay, T. Hovi, Proposals for the classification of human rhinovirus species C into genotypically assigned types, J. Gen. Virol. 91 (2010) 2409–2419.
[13] Picornaviridae Study Group. http://www.picornaviridae.com (accessed 02.09.14.).
[14] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (4) (1987) 406–425.
[15] M. Imakita, K. Shiraki, C. Yutani, H. Ishibashi-Umeda, Pneumonia caused by rhinovirus, Clin. Infect. Dis. 30 (2000) 611–612.
[16] D.J. Jackson, R.E. Gangnon, M.D. Evans, K.A. Roberg, E.L. Anderson, T.E. Pappas, et al., Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children, Am. J. Respir. Crit. Care. Med. 178 (2008) 667–672.
[17] R.R. Jansen, J. Wieringa, S.M. Koekkoek, C.E. Visser, D. Pajkrt, R. Molenkamp, et al., Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values, J. Clin. Microviol. 49 (7) (2011) 2631–2636.
[18] C.N. Camargo, E. Carraro, C.F. Granato, N. Bellei, Human rhinovirus infections in symptomatic and asymptomatic subjects, Braz. J. Microbiol. 43 (4) (2012) 1641–1645.
[19] T. Jartti, P. Lehtinen, T. Vuorinen, O. Koskenvuo Ruuskanen, Persistence of rhinovirus and enterovirus RNA after acute respiratory illness in children, J. Med. Virol. 72 (2004) 695–699.
[20] H. Harvala, I. Robertson, E.C. McWilliam Leitch, K.C. BenschopK. Wolthers, K. Templeton, P. Simmonds, Epidemiology and clinical associations of human parechovirus respiratory infections, J. Clin. Microbiol. 46 (10) (2008) 3446–3453.
[21] P. Kolehmainen, S. Oikarinen, M. Koskiniemi, O. Simell, J. Ilonen, M. Knip, H. Hyöty, S. Tauriainen, Human parechoviruses are frequently detected in stool of healthy Finnish children, J. Clin. Virol. 54 (2) (2012) 156–161.
[22] M. Hara, S. Takao, Y. Shimazu, T. Nishimura, Three-year study of viral etiology and features of febrile respiratory tract infections in Japanese pediatric outpatients, Pediatr. Infect. Dis. J. 33 (2014) 687–692.
[23] R. Brittain-Long, L.M. Andersson, S. Olofsson, M. Lindh, J. Westin, Seasonal variations of 15 respiratory agents illustrated by the application of a multiplex polymerase chain reaction assay, Scand. J. Infect. Dis. 44 (2012) 9–17.
[24] M.M. Kusel, N.H. de Klerk, P.G. Holt, T. Kebadze, S.L. Johnston, P.D. Sly, Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study, Pediatr. Infect. Dis. J. 25 (2006) 680–686.
[25] M.P. Fairchok, E.T. Martin, S. Chambers, J. Kuypers, M. Behrens, L.E. Braun, J.A. Englund, Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare, J. Clin. Virol. 49 (2010) 16–20.
[26] J.O. Wishaupt, A. Russcher, L.C. Smeets, F.G.A. Versteegh, N.G. Hartwig, Clinical impact of RT–PCR for pediatric acute respiratory infections: a controlled clinical trial, Pediatrics 128 (2011) e1113–e1120.
[27] K.E. Templeton, S.A. Scheltinga, M.F.C. Beersma, A.C.M. Kroes, E.C.J. Claas, Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza A and influenza B viruses: respiratory syncytial virus, and parainfluenza viruses 1,2,3, and 4, J. Clin. Microbiol. 42 (4) (2004) 1564–1569.
[28] G.R. Chidlow, G.B. Harnett, G.R. Shellam, D.W. Smith, An economical tandem multiplex real-time PCR technique for the detection of a comprehensive range of respiratory pathogens, Viruses 1 (2009) 42–56.
[29] A.C. van de Pol, A.M. van Loon, T.F.W. Wolfs, N.J.G. Jansen, M. Nijhuis, E.K. Breteler, R. Schuurman, J.W.A. Rossen, Increased detection of respiratory syncytial virus: parainfluenza viruses, and adenoviruses with real-time PCR in samples from patients with respiratory symptoms, J. Clin. Microbiol. 45 (7) (2007) 2260–2262.
[30] R. Mentel, U. Wegner, R. Bruns, L. Gürtler, Real-time to improve the diagnosis of respiratory syncytial virus infection, J. Med. Microbiol. 52 (2003) 893–896.
[31] F. Reymond, J. Carbonneau, N. Boucher, L. Robitaille, S. Boisvert, W. Wu, et al., Comparison of automated microarray detection with real-time PCR assays for detection of respiratory viruses in specimens obtained from children, J. Clin. Microbiol. 47 (3) (2009) 743–750.
[32] C. Tapparel, S. Cordey, S.V. Belle, L. Turin, W. Lee, N. Regamey, et al., New molecular detection tools adapted to emerging rhinoviruses and enteroviruses, J. Clin. Microbiol. 47 (6) (2009) 1742–1749.
[33] F. Neske, K. Blessing, F. Tollmann, J. Schubert, A. Rethwilm, H.W. Kreth, Weissbrich B. Real-time, for diagnosis of human bocavirus infections and phylogenetic analysis, J. Clin. Microbiol. 45 (7) (2007) 2116–2122.
[34] W. Nix, K. Maher, E.S. Johansson, B. Niklasson, A.M. Lindberg, M.A. Pallansch, M.S. Oberste, Detection of all known parechoviruses by real-time PCR, J. Clin. Microbiol. 46 (8) (2008) 2519–2524.
[35] S. Wong, K. Pabbaraju, X.L. Pang, B.E. Lee, J.D. Fox, Detection of a broad range of human adenoviruses in respiratory tract samples using a sensitive multiplex real-time PCR assay, J. Med. Virol. 80 (5) (2008) 856–865.
[36] M. Nakauchi, Y. Yasui, T. Miyoshi, H. Minagawa, T. Tanaka, M. Tashiro, T. Kageyama, One-step real-time reverse transcription-PCR assays for detecting and subtyping pandemic influenza A/H1N1 2009, seasonal influenza A/H1N1, and seasonal influenza A/H3N2 viruses, J. Virol. Methods 171 (2011) 156–162.
[37] M. Nakauchi, I. Takayama, H. Takahashi, K. Oba, H. Kubo, A. Kaida, M. Tashiro, T. Kageyama, Real-time RT–PCR assays for discriminating influenza B virus Yamagata and Victoria lineages, J. Virol. Methods 205 (2014) 110–115.

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Seasonal variations of respiratory viruses and etiology of human rhinovirus infection in children

Saeko Morikawa[a,*], Urara Kohdera[b], Taisuke Hosaka[b], Kousuke Ishii[b], Shohei Akagawa[b], Satoshi Hiroi[a], Tetsuo Kase[a]

[a] Division of Virology, Department of Infectious Diseases, Osaka Prefectural Institute of Public Health, 1-3-69, Nakamichi, Higashinari-ku, Osaka, 537 0025, Japan
[b] Nakano Children's Hospital, 4-13-17, Shinmori, Asahi-ku, Osaka, 535 0022, Japan

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ABSTRACT

Background: Using the polymerase chain reaction (PCR) method it is possible to detect uncultivable viruses and discover multiple viral infections. However, the clinical importance of these findings in relation to symptoms is not known.
Objectives: The seasonal fluctuations of respiratory viruses and the clinical outcomes of single infections and dual infections were investigated.
Study design: Nasal aspirate samples were obtained from outpatients and inpatients of a children's hospital and these samples were subjected to real-time PCR to detect 16 respiratory viruses. Seasonal variations of the 16 viruses and the clinical outcomes such as wheezing, the need for oxygenation and prolonged hospitalization of patients with single viral infections and multiple infections were determined for the 5 most often detected viruses.
Results: Among 512 specimens analyzed, one or more viruses were detected in 424 (83%) specimens. Two or more viruses were detected in 160 samples (31% of all samples). The epidemic peaks of the viruses did not coincide with each other. Rhinoviruses were the most frequently detected viruses and their coinfection rates were also higher. However, the disease severity in the lower respiratory tract did not differ in most respiratory viral infections regardless of whether there was single infection or dual infection with a rhinovirus and other respiratory virus.
Conclusions: Seasonal distribution was seen for each virus. There were no significant differences in clinical symptoms in the children studied. Because the infection of rhinoviruses is the common occurrence in children, it is hypothesized that the factors related to disease severity are mainly the underlying conditions of the children.

1. Background

Respiratory tract infections are frequently seen in children and a significant number of these infections are caused by viral pathogens [1,2]. Especially for infants, viral respiratory infections carry a high risk for severe symptoms resulting in hospitalization. There is a strong correlation between viral bronchiolitis in infants and wheezing later in childhood [3]. However, most children show mild symptoms during viral respiratory infections involving only the nose and upper respiratory passages. Moreover, clinically useful antivirals do not exist for most such viruses and it is thought that for viral respiratory infections it is not necessary to examine the pathogen.

Recently, nucleic acid amplification tests such as PCR are increasingly being used to diagnose viral respiratory tract infections. Several studies have shown that most common respiratory viruses have epidemic seasons in many areas [4,7]. PCR makes it possible to detect uncultivable viruses such as human bocavirus and rhinovirus C and discover concurrent viral infections. However, the clinical importance of these findings with regard to symptoms is not known. Some reports indicate that human "classical" subtypes

Abbreviations: PCR, polymerase chain reaction; RS virus, respiratory syncytial virus.

* Corresponding author.
E-mail addresses: morikawa@iph.pref.osaka.jp (S. Morikawa), kohdera@nakano-kodomo.or.jp (U. Kohdera), thosaka@pd6.so-net.ne.jp (T. Hosaka), kousuke.22.ishii@gmail.com (K. Ishii), shohei@mbk.nifty.com (S. Akagawa), hiroi@iph.pref.osaka.jp (S. Hiroi), kasetetsuo@iph.pref.osaka.jp (T. Kase).

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Table 1
The monthly variation of viruses detected in nasal aspirates during the study period.

Virus[a]	Month													Total
	2013-April	May	June	July	August	September	October	November	December	2014-January	February	March	April	*n* (%)
Parainfluenzavirus 1	0	0	4	4	2	1	3	0	1	0	0	0	1	16(3.1)
Parainfluenzavirus 2	0	0	0	1	0	0	0	0	0	0	0	0	0	1(0.2)
Parainfluenzavirus 3	5	17	9	6	2	0	0	0	0	0	0	0	2	41(8.0)
Parainfluenzavirus 4	0	0	1	3	8	2	0	0	0	0	0	0	0	14(2.7)
RSvirus	2	3	3	3	3	6	8	7	5	0	2	8	2	52(10.2)
human Metapneumovirus	3	4	1	5	2	0	0	0	0	1	14	21	17	68(13.3)
Enterovirus/Rhinovirus	6	27	21	24	13	15	19	22	20	5	13	16	18	219(42.8)
human Bocavirus	4	20	9	4	1	0	0	1	1	0	2	10	12	64(12.5)
human Parechovirus	0	2	0	2	3	3	3	3	2	0	1	0	0	19(3.7)
Adenovirus	2	15	10	6	4	2	5	9	10	3	4	6	10	86(16.8)
human Coronavirus OC43	1	1	3	0	0	0	0	0	1	1	0	1	0	8(1.6)
human Coronavirus NL63	0	1	0	0	0	0	0	0	0	0	1	5	1	8(1.6)
human Coronavirus 229E	0	0	1	1	0	0	0	0	0	0	1	0	1	4(0.8)
human Coronavirus HKU-1	0	1	0	0	1	0	0	0	2	2	1	1	0	8(1.6)
Influenza virus type A	0	1	0	0	0	0	0	1	0	2	5	2	0	11(2.1)
Influenza virus type B	0	1	1	0	0	0	0	0	1	0	2	1	6	12(2.3)
Total positive viruses	23	93	63	59	39	29	38	43	43	14	46	71	70	631
Enterovirus	0	3	1	1	3	5	4	3	2	1	2	0	0	25(4.9)
Rhinovirus A	6	15	7	20	7	10	9	5	9	4	2	11	8	113(22.1)
Rhinovirus B	0	1	2	0	0	0	1	0	1	0	1	1	1	8(1.6)
Rhinovirus C	0	7	7	3	3	0	5	14	9	0	9	4	10	71(13.9)
EV/RV Untyped	0	2	4	0	1	0	0	1	0	0	0	0	0	8(1.6)
Samples with 1 virus	3	31	22	24	15	17	28	21	16	8	21	19	27	262(51.2)
Samples with 2 viruses	7	22	17	10	8	3	5	7	11	3	6	8	11	118(23.0)
Samples with 3 or more viruses	2	6	2	5	3	2	0	3	2	0	4	8	7	44(8.6)
Total samples	15	69	45	46	32	26	38	37	36	20	38	55	55	512 (100)

RS virus, respiratory syncytial virus.
[a] Multiple viruses included.

of coronavirus, OC43, NL63, 229E and HKU-1, have low impacts on respiratory health [8,9].

2. Objectives

In this study, separate real-time PCR assays were used to detect 16 respiratory viruses in nasal aspirates taken from pediatric patients and we investigated the seasonal fluctuations of the respiratory viruses. We also compared the clinical outcomes such as wheezing, the need for oxygenation and prolonged hospitalization, for patients with single and multiple viral infections.

3. Study design

3.1. Patients and samples

From week seventeen 2013 to week sixteen 2014, nasal aspirate samples were obtained from outpatients and inpatients of a children's hospital. Their symptoms were systematically recorded by the attending physicians. Written informed consent was obtained from the parents. Of the 513 samples obtained, 1 specimen was excluded because of withdrawal of approval.

The median age of the patients was 1y (range 0–14 years). Age groups were: 0 year 35.9% (n = 184), 1 year 32.4% (n = 166), 2 years 11.9% (n = 61), 3years 7.2% (n = 37), 4 years 5.6% (n = 30), and ≥5 years 6.8% (n = 35). The proportion of females was 41.4%.

3.2. Molecular analysis

Each sample was amplified using primers and probes specific for each of the targets as previously described [10]. Briefly, nucleic acids were extracted from 200 μL specimens using the Magtration System with a MagDEA viral DNA/RNA 200 kit (Precision System Science Co., Ltd., Chiba, Japan) with a 50 μL elution volume. RT reactions were performed using a ReverTra Ace qPCR RT kit (Toyobo Co., Ltd., Osaka, Japan) following the manufacturer's instructions. The cDNA was then amplified using Realtime PCR Master Mix (Toyobo) with a total volume of 25 μL. The sensitivity of each of the real-time PCR methods was reported previously [10]. Enteroviruses and rhinoviruses were genotyped by direct sequencing. Amplification of the VP4/VP2 region of the enterovirus or rhinovirus for typing was performed with semi-nested RT-PCR as previously described [11]. The purified PCR products were subjected to direct sequencing with a BigDye Terminator v1.1 kit as per the manufacturer's instructions (Applied Biosystems, CA, USA). Sequence analysis was performed using the DNADynamo program (Blue Tractor Software, UK). Using MEGA5.2 (Tamura et al., 2011, Ver5.2.2), we employed the neighbor-joining method [12] to construct phylogenetic trees from the VP4/VP2 region (420 nt) sequences of prototype isolates of each rhinovirus type commonly used in epidemiologic studies of human rhinoviruses retrieved from GenBank [13–15] and new types proposed previously [13,16,17]. Genotypes were assigned on the basis of their clustering with known prototype reference strains.

3.3. Statistics

The Kruskal–Wallis test, Mann–Whitney U-test and Fisher's exact test were used for comparisons. For all analyses, a p-value of less than 0.05 was considered significant. Statistical analysis was performed using SPSS v16.0 (SPSS Inc., Tokyo, Japan).

4. Results

4.1. Real-time PCR detection

Among the 512 specimens analyzed, one or more viruses were detected in 424 (83%) specimens (Table 1). Two or more viruses were detected in 160 samples (31% of all samples). Only one specimen included 5 distinct viruses (human metapneumovirus,

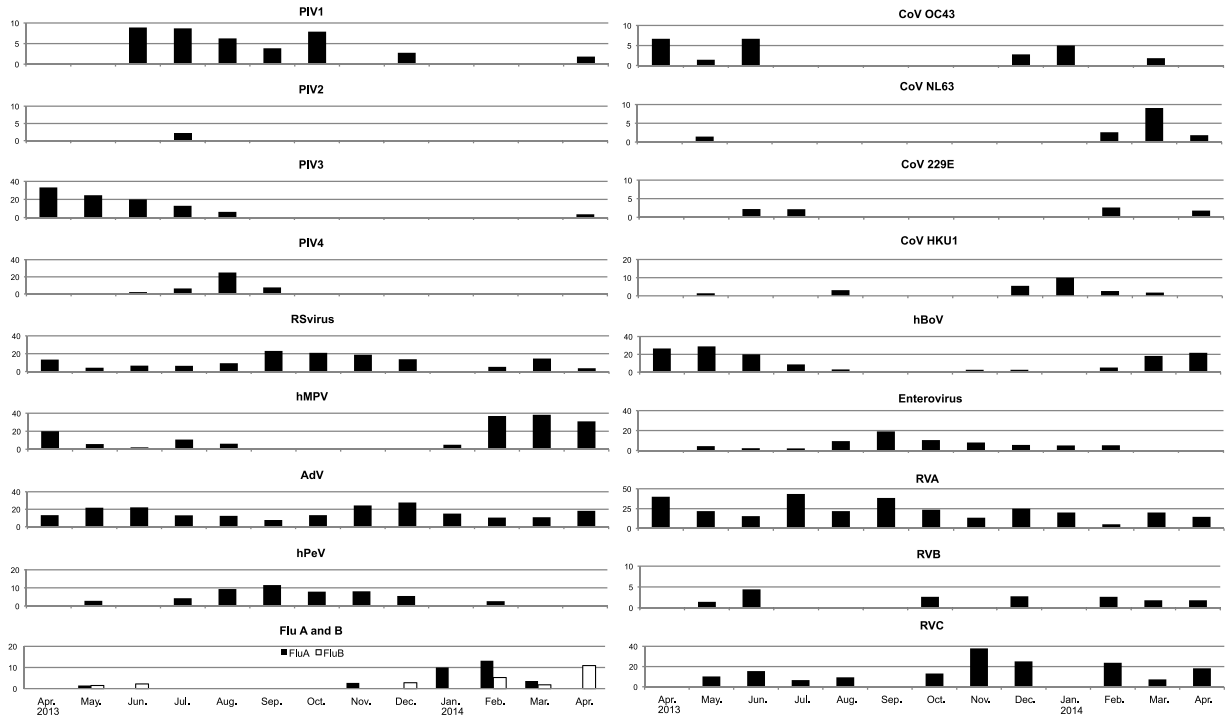

Fig. 1. Monthly prevalences of detection of 19 respiratory viruses/serotypes by real-time PCR assay from week 17, 2013 to week 16, 2014. (percent positive).
Note that the scale on the vertical axis differs between viruses.
PIV, parainfluenza viruses; RS virus, respiratory syncytial virus; hMPV, human metapneumovirus; AdV, adenovirus; hPeV, human parechovirus, Flu A and B, influenzavirus type A and type B;
CoV, coronavirus; hBoV, human bocavirus; RVA, rhinovirus A; RVB, rhinovirus B; RVC, rhinovirus C.

Coxsackievirus type B5, rhinovirus C, bocavirus and influenza virus type A).

Rhinoviruses were found most often (n = 192, 37.5% of all samples and 45.3% of positive samples) followed by adenoviruses (n = 86, 16.8% of all samples) and human metapneumovirus (n = 68, 13.3%).

4.2. Seasonal distribution

Influenza virus types A and B were detected in the winter and human metapneumovirus was detected during the spring months. Human bocavirus and parainfluenza virus type 3 were found during the spring and early summer. Parainfluenza virus type 1 and parechovirus were detected mainly in the summer. The detection of RS virus increased in the autumn.

Genetically conserved regions of both enteroviruses and rhinoviruses were detected by real-time PCR all year round with a high proportion of positive samples. Genotyping revealed the presence of enteroviruses in the summer and a decrease in rhinovirus A in the winter. On the other hand, rhinovirus C was detected in the winter months. Adenoviruses were detected mainly in the summer and winter (Fig. 1).

4.3. Multiple infections

Next, we evaluated the prevalence of multiple infections by the viruses. For the human bocavirus, parechovirus and rhinoviruses A and C, the rates of coinfection were high compared with other respiratory viruses. Rhinoviruses were the most frequently detected viruses and their coinfection rates were also higher than those of the other viruses. Therefore, we compared the clinical symptoms caused by five types of viruses, adenoviruses, human bocavirus, RS virus, parainfluenza virus type 3, and human metapneumovirus, which were detected most often after rhinoviruses, and rhinovirus single infections and symptoms in cases with dual infections including rhinoviruses.

There was no significant difference between the number of days of hospitalization caused by rhinoviruses and the other five viruses. The number of days in the hospital of patients in whom RS virus was detected was longer than that of patients infected with human metapneumovirus (Table 2).

For the five viruses discussed above, we compared the number of hospitalization days of the cases with single infections by the each 5 viruses with those having dual infections with a rhinovirus and those with dual infection with a virus other than a rhinovirus. The number of days of hospitalization of the children with parainfluenza virus type 3 infection alone was shorter than for children with paranfluenza virus and rhinovirus dual infection. On the other hand, children with infection by human metapneumovirus alone spent fewer days in the hospital than those with dual infections by human metapneumovirus and a respiratory virus other than a rhinovirus (Table 3).

We next compared the requirement for oxygenation and the presence of wheezing of the children with single infections and dual infections with a rhinovirus or other respiratory virus. The patients with dual infections with an adenovirus and rhinovirus needed significantly more oxygenation than those with an adenovirus infection or dual infection with an adenovirus and other respiratory virus. However, the severity of the lower respiratory tract disease for which the requirement of oxygenation was assumed and the presence of wheezing as an index did not differ among most respiratory viral infections, regardless of whether they were single

Table 2
Asymptotic *p*-values of duration of hospitalization caused by single infection with each of six viruses.

	PIV3	RSV	hMPV	hBoV	AdV	RV
No. of single detections (% of total)	15 (36.6)	26 (50)	37 (54.4)	12 (18.8)	24 (27.9)	98 (49.0)
Duration of hospitalization, median number of days	8	9	7	7.5	7.5	8
PIV3		0.310	0.576	0.901	0.930	0.864
RSV			**0.048***	0.408	0.618	0.150
hMPV				0.661	0.440	0.599
hBoV					0.747	0.961
AdV						0.685

Mann–Whitney U test. asymptotic significance (2-tailed).
* $p < 0.05$. No other significant between-group differences.

Table 3
Comparison of single and dual infections with and without rhinoviruses by duration of hospitalization.

	PIV3	RSV	hMPV	hBoV	Ad
Total number of detections	41	52	68	64	86
No. of single detections (% of total)	15 (36.6)	26 (50)	37 (54.4)	12 (18.8)	24 (27.9)
Duration of hospitalization, median no. of days	8	9	7	7.5	7.5
Dual detection with rhinovirus: No. (%)	6 (14.6)	10 (19.2)	7 (10.3)	8 (12.5)	18 (20.9)
Duration of hospitalization, median no. of days	12	8	7	7.5	9
asymptotic significance (2-tailed)	**0.030***	0.886	0.742	0.907	0.099
Dual detection with other respi. Virus: No. (%)	11 (26.8)	9 (17.3)	8 (11.8)	21 (32.8)	19 (22.1)
Duration of hospitalization, median no. of days	8	9	10	9	9
asymptotic significance (2-tailed)	0.213	0.338	**0.009***	0.384	0.094
Dual detection with rhinovirus vs. dual detection with other respiratory virus Asymptotic significance (2-tailed)	0.245	0.432	0.144	0.431	0.939

Mann–Whitney U test.
* $p < 0.05$. No other significant between-group differences.

Table 4
Correlations between coinfection with a rhinovirus or other respiratory virus and wheezing and oxygen treatment.

Outcome of interest	Factors	Wheezing			Oxgen		
		p-values	OR	95% CI	*p*-values	OR	95% CI
PIV3	RV coinfection[b]	1.0000	1.25	0.10–15.11	0.1196	8.00	0.96–66.95
	other virus coinfection[c]	0.4065	0.44	0.08–2.55	0.4065	2.29	0.39–13.33
RV coinfection vs. other virus coinfection		0.6000	2.86	0.24–33.90	0.3348	3.50	0.43–28.45
RSV	RV coinfection	1.0000	0.86	0.17–4.28	0.1186	5.14	0.71–37.15
	other virus coinfection	0.6936	0.74	0.14–3.78	0.0946	6.00	0.81–44.35
RV coinfection vs. other virus coinfection		1.0000	1.17	0.17–8.09	1.0000	0.86	0.12–5.94
hMPV	RV coinfection	0.6746	0.64	0.12–3.32	0.5934	2.07	0.32–13.25
	other virus coinfection	1.0000	1.44	0.25–8.22	0.3262	3.10	0.58–16.59
RV coinfection vs. other virus coinfection		0.6084	0.44	0.05–3.98	1.0000	0.67	0.08–5.88
BoV	RV coinfection	1.0000	1.19	0.19–7.46	1.0000	1.20	0.19–7.77
	other virus coinfection	0.4334	2.29	0.50–10.50	0.6905	0.63	0.13–2.99
RV coinfection vs. other virus coinfection		0.6460	0.52	0.09–2.99	0.6460	1.92	0.33–11.03
AdV	RV coinfection	0.1038	3.57	0.81–15.71	**0.0114**[a]	**5.97**	**1.52–23.43**
	other virus coinfection	0.1991	2.68	0.68–10.53	0.4947	1.75	0.44–6.98
RV coinfection vs. other virus coinfection		1.0000	1.33	0.25–7.01	0.1031	3.40	0.88–13.19
RVA vs. RVB[d]		0.6258	0.58	0.07–4.43	1.0000	0.45	0.02–9.02
RVA vs. RVC		0.6633	1.28	0.54–3.05	0.4626	1.49	0.56–3.95
RVB vs. RVC		0.5868	2.23	0.28–17.61	0.5588	3.29	0.16–65.92

[a] $p < 0.05$. No other significant between-group differences.
[b] single infection vs. dual infection with a rhinovirus (RV).
[c] single infection vs. dual infection with respiratory viruses other than rhinoviruses.
[d] comparison of clinical severity of single infections by rhinoviral genogroups.

infections or dual ones with a rhinovirus and other respiratory virus (Table 4).

5. Discussion

In this study, separate real-time PCR assays were used to detect 12 RNA viruses and two DNA viruses, and real-time reverse transcription (RT) PCR was used to detect influenza viruses A and B. Of the 512 samples analyzed, 424 were positive 1 virus or more. The overall viral detection rate was 83%, which was much higher than in similar past reports [5–7,18]. The reason may be that our method had many detection targets. Furthermore, the higher detection rates among young children likely correspond to the higher incidence of viral respiratory tract infections in children, although other factors such as pre-existing immunity might also have played a role [4]. Since the specimens from children aged 1 year or younger accounted for 68% of those studied, it is considered that the rate of viral detection and the rate of concurrent infections became higher than in past reference data. The largest number of viruses detected in one sample was 5 in the nasal aspirate from a 9-month-old girl. When comparing the number of viruses detected per specimen,

it was found that the specimens from younger patients tended to include more than one virus (data not shown).

Seasonal distribution was seen for each virus. The epidemic peak of each virus was about the same as in another report from Japan [19]. Seasonal influenza virus type A migrates globally between epidemics and is reintroduced every winter season in temperate climates [20], although the underlying cause of the seasonality of the other respiratory viruses remains unknown. It has been suggested that rhinovirus infections could reduce subsequent RS virus and influenza virus type A infections by inducing an interferon response, thereby creating an undesirable environment for these viruses [21,22]. In this study, the peaks for the various viral epidemics did not coincide. Thus, it is thought that some kind of interference by viruses may influence epidemics of respiratory viruses.

In this study, human rhinoviruses were the most common viruses. Rhinoviruses are thought to be mainly associated with the common cold, causing mild respiratory symptoms [23]. These viruses are classified into three species and divided into more than 160 serotypes or genotypes. Thus they are among the mostly commonly detected viruses in respiratory specimens of children [10]. However, recent reports suggest that rhinovirus infections may induce and/or exacerbate asthma and be responsible for lower respiratory tract infections with severe symptoms [24,25].

Based on the sequence data, rhinovirus C was detected mainly in the winter, whereas rhinovirus A was detected all year round, with a high proportion of positive samples in June (44% of the samples). Although rhinovirus B was detected, its seasonality was not clear. However, it became clear that there was a difference in the epidemic seasons of rhinoviruses A and C. Furthermore, the detected rhinoviruses consisted of 32 genotypes of group A, 5 genotypes of group B and 21 genotypes of group C, suggesting that multiple genotypes were brought into the area and that epidemics of some of them might occur at the same time (Supplementary Table 1). Rhinovirus A consists of 80 serotypes and B consists of 32 types, including genotypes, and there are now 55 rhinovirus C genotypes proposed [17]. It is not clear whether the genotypes of the rhinoviruses detected in this study cause severe illness.

We also compared the clinical symptoms of single infections and dual infections by rhinoviruses and other respiratory viruses of the children infected by one of the five most commonly detected respiratory viruses. The results revealed that there were no significant differences in the number of days of hospitalization, the necessity for oxygen inhalation or the existence of wheezing between the children with single infections and those with dual infections. In former reports that evaluated the impacts of rhinoviruses on lower respiratory infections, there were only marginal differences between the different rhinovirus groups and between single rhinovirus infection and rhinovirus coinfection [26,27]. Though there was no significant difference in the number of hospitalization days of patients with single infections by rhinoviruses or other respiratory viruses, our data suggested the importance of rhinoviruses as a potential cause of pediatric pneumonia. Recently, our group evaluated the prevalence of rhinovirus infections among asymptomatic children [10]. Rhinoviruses were often detected in their throats at a time without any symptoms. Since rhinoviruses do not exist in the upper respiratory tract for a long time even if a child does not show symptoms, these were “active” asymptomatic infections rather than persistent infections.

In conclusion, rhinoviruses are causative agents of various conditions ranging from asymptomatic infection to lower respiratory tract infection and pneumonia. Rhinovirus coinfection with other respiratory viruses is not responsible for more severe symptoms, so it is hypothesized that the factors related to disease severity are mainly the underlying conditions of the children.

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Competing interest

None declared.

Ethical approval

This study was approved by the Osaka Prefectural Institute of Public Health ethical committee (No. 1302-05-01).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2015.10.001.

References

[1] J.S. Tregoning, J. Schwarze, Respiratory viral infection in infants: causes, clinical symptoms, virology, and immunology, Clin. Microbiol. Rev. 23 (2010) 74–98.
[2] C. Cabello, M.E. Manjarrez, R. Olvera, J. Villalba, L. Valle, I. Paramo, Frequency of viruses associated with acute respiratory infections in children younger than five years of age at a locality of Mexico City, Mem. Inst. Oswaldo Cruz 101 (2006) 21–24.
[3] E. Piippo-Savolainen, M. Korppi, Wheezy babies—wheezy adults? Review on long-term outcome until adulthood after early childhood wheezing, Acta Pediatr. 97 (2008) 5–11.
[4] R. Brittain-Long, L.M. Anderson, S. Olofsson, M. Lindh, J. Westin, Seasonal variations of 15 respiratory agents illustrated by the application of a multiplex polymerase chain reaction assay, Scand. J. Infect. Dis. 44 (2012) 9–17.
[5] J.O. Wishaupt, A. Russcher, L.C. Smeets, F.G.A. Versteegh, N.G. Hartwig, Clinical impact of RT-PCR for pediatric acute respiratory infections: a controlled clinical trial, Pediatrics 128 (2011) e1113–e1120.
[6] M.S. Al-Ayed, A.M. Asaad, M.A. Qureshi, M.S. Ameen, Viral etiology of respiratory infections in children in southwestern Saudi Arabia using multiplex reverse-transcriptase polymerase chain reaction, Saudi Med. J. 35 (2014) 1348–1353.
[7] J.A. Weigl, W. Puppe, C.U. Meyer, R. Berner, J. Forster, H.J. Schumitt, F. Zepp, Ten years' experience with year-round active surveillance of up to 19 respiratory pathogens in children, Eur. J. Pediatr. 166 (2007) 957–966.
[8] Q. Lepiller, H. Barth, F. Lefebvre, R. Herbrecht, P. Lutz, R. Kessler, S. Fafi-Kremer, F. Stoll-Keller, High incidence but low burden of coronaviruses and preferential associations between respiratory viruses, J. Clin. Microbiol. 51 (2013) 3039–3046.
[9] M.M. Prill, M.K. Iwane, K.M. Edwards, J.V. Williams, G.A. Weinberg, M.A. Staat, et al., Human coronavirus in young children hospitalized for acute respiratory illness and asymptomatic controls, Pediatr. Infect. Dis. J. 31 (2012) 235–240.
[10] S. Morikawa, S. Hiroi, T. Kase, Detection of respiratory viruses in gargle specimens of healthy children, J. Clin. Virol. 64 (2015) 59–63.
[11] H. Ishiko, Y. Shimada, M. Yonaha, O. Hashimoto, A. Hayashi, K. Sakae, N. Takeda, Molecular diagnosis of human enteroviruses by phylogeny-based classification by use of the VP4 sequence, J. Infect. Dis. 185 (2002) 744–754.
[12] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425.
[13] C.L. McIntyre, N.J. Knowles, P. Simmonds, Proposals for the classification of human rhinovirus species A, B and C into genotypically assigned types, J. Gen. Virol. 94 (2013) 1791–1806.
[14] C. Henquell, A. Mirand, A.L. Deusebis, C. Regagnon, C. Archimbaud, M. Chambon, et al., Prospective genotyping of human rhinoviruses in children and adults during the winter of 2009–2010, J. Clin. Virol. 53 (2012) 280–284.
[15] A. Kaida, H. Kubo, K. Takakura, M. Togawa, M. Shiomi, U. Koudera, N. Iritani, Molecular epidemiology of human rhinovirus C in patients with acute respiratory tract infections in Osaka City, Japan, Jpn. J. Infect. Dis. 64 (2011) 488–492.

[16] P. Simmonds, C. McIntyre, C. Savolainen-kopra, C. Tapparel, I.M. Mackay, T. Hovi, Proposals for the classification of human rhinovirus species C into genotypically assigned types, J. Gen. Virol. 91 (2010) 2409–2419.
[17] Picornaviridae study group. http://www.picornaviridae.com (accessed 13.07.15.).
[18] J. Kuypers, N. Wright, J. Ferrenberg, M.-L. Huang, A. Cent, L. Corey, R. Morrow, Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children, J. Clin. Microbiol. 44 (2006) 2382–2388.
[19] H. Hamada, A. Ogura, C. Hotta, T. Wakui, T. Ogawa, M. Terai, Epidemiological study of respiratory viruses detected in patients under two years old who required admission because of lower respiratory disease, Kansenshogaku Zassi 88 (2014) 423–429.
[20] M.I. Nelson, L. Simonsen, C. Viboud, M.A. Miller, E.C. Holmes, Phylogenetic analysis reveals the global migration of seasonal influenza A viruses, PloS Pathog. 3 (2007) e131.
[21] R.M. Greer, P. McErlean, K.E. Arden, C.E. Faux, A. Nitsche, S.B. Lambert, et al., Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections? J. Clin. Virol. 45 (2009) 10–15.
[22] A. Linde, M. Rotzen-Ostlund, B. zweygberg-Wirgart, S. Rubinova, M. Brytting, Does viral interference affect spread of influenza? Euro Surveill. 14 (2009) pii19354.
[23] A. Ruohola, M. Waris, T. Allander, T. Ziegler, T. Heikkinen, O. Ruuskanen, Viral etiology of common cold in children, Finland Emerg. Infect. Dis. 15 (2009) 344–346.
[24] D.J. Jackson, R.E. Gangnon, M.D. Evans, K.A. Roberg, E.L. Anderson, T.E. Pappas, M.C. Printz, et al., Wheezing rhinovirus illness in early life predict asthma development in high-risk children, Am. J. Respir. Crit. Care Med. 178 (2008) 667–672.
[25] J.E. Gern, The ABCs of rhinoviruses, wheezing and asthma, J. Virol. 84 (2010) 7418–7426.
[26] S. Esposito, C. Daleno, C. Tagliabue, A. Scala, R. Tenconi, I. Borzani, E. Fossali, C. Pelucci, A. Piralla, N. Principi, Impact of rhinoviruses on pediatric community-acquired pneumonia, Eur. J. Clin. Microbiol. Infect. Dis. 31 (2012) 1637–1645.
[27] N.T. Paula, B.M. Carneiro, J. Yokosawa, G.R.O. Freitas, T.F.M. Olivaira, L.F. Costa, H.L. Silveira, D.A.O. Queiroz, Human rhinovirus in the lower respiratory tract infections of young children and the possible involvement of a secondary respiratory viral agent, Mem. Inst. Oswaldo Cruz 106 (2011) 316–321.

RESEARCH PAPER

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Detection of influenza vaccine effectiveness among nursery school children: Lesson from a season with cocirculating respiratory syncytial virus

Keiko Nakata[1,2,*], Megumi Fujieda[3], Hitoshi Miki[1], Wakaba Fukushima[1], Satoko Ohfuji[1], Akiko Maeda[1], Tetsuo Kase[2], and Yoshio Hirota[1]

[1]Department of Public Health; Osaka City University Graduate School of Medicine; Osaka, Japan; [2]Department of Infectious Diseases; Osaka Prefectural Institute of Public Health; Osaka, Japan; [3]Department of Neuropsychiatry; Kurume University School of Medicine; Fukuoka, Japan

Keywords: case classification, influenza-like illness (ILI), influenza vaccine effectiveness, observation period, outcome misclassification, RSV infection

In the winter influenza epidemic season, patients with respiratory illnesses including respiratory syncytial virus (RSV) infections increase among young children. Therefore, we evaluated the effectiveness of influenza vaccine against influenza-like illness (ILI) using a technique to identify outbreaks of RSV infection and to distinguish those patients from ILI patients. The study subjects were 101 children aged 12 to 84 months attending nursery school. We classified the cases into 6 levels based on the definitions of ILI for outcomes. We established observation periods according to information obtained from regional surveillance and rapid diagnostic tests among children. Multivariate odds ratios (ORs) for each case classification were obtained using a logistic regression model for each observation period. For the entire observation period, ORs for cases with fever plus respiratory symptoms were reduced marginally significantly. For the local influenza epidemic period, only the OR for the most serious cases was significantly decreased (0.20 [95%CI: 0.04-0.94]). During the influenza outbreak among the nursery school children, multivariate ORs for fever plus respiratory symptoms decreased significantly ($\geq$ 38.0°C plus $\geq$ one symptoms: 0.23 [0.06-0.91], $\geq$ 38.0°C plus $\geq$ 2 symptoms: 0.21 [0.05-0.85], $\geq$ 39.0°C plus $\geq$ one symptoms: 0.18 [0.04-0.93] and $\geq$ 39.0°C plus $\geq$ 2 symptoms: 0.16 [0.03-0.87]). These results suggest that confining observation to the peak influenza epidemic period and adoption of a strict case classification system can minimize outcome misclassification when evaluating the effectiveness of influenza vaccine against ILI, even if influenza and RSV cocirculate in the same season.

Introduction

Annual morbidity associated with influenza is highest among young children, for whom the rate of hospitalization has been estimated to be 1 per 1000 children aged under 5 years.[1] Therefore, many studies have investigated the efficacy of influenza vaccines among young children.[2-10] However, the results of these studies were not consistent because influenza epidemics vary a great deal depending on the time, place and population.[11] Accordingly, confining the subjects to true influenza patients is a key point for minimizing outcome misclassification.

In the winter, there are doubts about whether ILI patients have influenza because the infectious seasons of influenza and other respiratory viruses overlap, compounding the clinical difficulty in distinguishing these illnesses.

Respiratory syncytial virus (RSV) infection is a typical respiratory tract infection among young children in the winter,[12-14] although the number of patients with RSV infection is smaller than that of influenza patients. However, outbreaks often occur among communal populations in households, nursery schools and inpatient facilities.[15-18] Therefore, detection of an outbreak among a specific population is difficult using only information obtained from a regional surveillance system. When the effectiveness of influenza vaccine against ILI is evaluated in an epidemiological study, it is critically important to differentiate patients with RSV infection from ILI patients in the influenza epidemic period.

On the basis of virus isolation data, respiratory viruses from ILI patients had been shown a characteristic seasonal pattern where the peaks of the influenza virus and RSV were distinct from each other.[19] However, some overlap of endemicity have been clearly demonstrated by nucleic acid-based diagnostic methods in the influenza season.[20-22] Therefore, to correctly assess the effectiveness of influenza vaccine, it is critical to exclude RSV

*Correspondence to: Keiko Nakata; Email: knakata@iph.pref.osaka.jp
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patients. It took some years to come to this conclusion because above key finding was reported during recent years.

The nursery school children studied here were a suitable cohort to verify the effectiveness of influenza vaccine because their chances for exposure to influenza viruses were relatively homogeneous. Additionally, they were always under observation by school nurses or their guardians, so there was a greater likelihood that their illness could be determined precisely.

Accordingly, we evaluated effectiveness of influenza vaccine against ILI in a season with cocirculating RSV among nursery school children using collected 2006-07 influenza season. In this study, we attempted to minimize outcome misclassification caused by ambiguous definition of the influenza epidemic period and case classification by using regional surveillance information and rapid diagnostic tests of the nursery school children.

Results

The subjects available for analysis were 101 children (45 children who were vaccinated twice, and 56 children who were not vaccinated). Ten children who were vaccinated once were excluded. The characteristics of vaccinees and nonvaccinees are compared in **Table 1**. Males were more frequent in the vaccinated group and the mean of age in months was higher in the unvaccinated group; however, there were no significant differences among these variables between vaccinees and nonvaccinees. Children who had asthma as an underlying illness, were vaccinated during the previous season, had a smoker in the family and had a vaccinated family were significantly more numerous in the vaccinated group. The children who slept longer and had more floor space per person were also more numerous in the vaccinated group. On the other hand, the number of family members was higher with marginal significance in the unvaccinated group.

The effectiveness of the vaccine for each outcome indicator during the entire observation period is shown in **Table 2**. In multivariate analysis, ORs for FaS, FaSS, FbS and FbSS were decreased, but not significance. For case definitions of ILI see Materials and Methods.

The effectiveness of the vaccine during the outbreak of RSV infection among the nursery schoolchildren is shown in **Table 3**. There were no significant decreases in ORs for any of the outcome indicators in either univariate or multivariate analysis.

The effectiveness of the vaccine during the local influenza epidemic period is shown in **Table 4**. The multivariate analysis revealed that vaccination was effective at preventing; FaSS and FbS with at least marginal significance (FaSS: 0.25 [0.06-1.01] and FbS: 0.22 [0.05-1.00]); however, the OR for FbSS, which was the most serious case classification among the outcome indicators, showed a significant decrease [0.20 (0.04-0.94]. Moreover, univariate and multivariate ORs for the outcome indicators **were all less than 1. The point estimates for** the defined levels of fever (38.0°C and 39.0°C), decreased gradually as the outcome classifications became more serious (Fa: 0.64, FaS: 0.31, FaSS: 0.25; or Fb: 0.53, FbS: 0.22, FbSS: 0.20). Additionally, when Fa and Fb, FaS and FbS, and FaSS and FbSS were compared, all point estimates decreased in the cases with higher fever levels.

Table 5 shows the effectiveness of the vaccine during the influenza outbreak among the nursery school children. The multivariate ORs for all outcome indicators were lower than the ORs in the other periods (**Tables 2–4**), and the ORs for FaS, FaSS, FbS and FbSS reached statistically significant levels (FaS: 0.23 [0.06-0.91]; FaSS: 0.21 [0.05-0.85]); FbS: 0.18 [0.04-0.93]; and FbSS: 0.16 [0.03-0.87]). For the local influenza epidemic period (**Table 4**), these point estimates for each defined level of fever (38.0°C and 39.0°C) also decreased gradually as the outcome classification level increased. All point estimates for outcome indicators decreased in the higher fever level. In multivariate analysis, it was found that the ORs for all outcome indicators

Table 1. Baseline characteristics of vaccinees and nonvaccinees

Characteristics	Vaccinee (N=45)	Nonvaccinee (N=56)	*P* value[1]
Sex (male)	29 (64)	28 (50)	0.146
Age (months)	55.0 (17.5–81.2)	58.6 (12.5–81.1)	0.710
Current body weight (kg)	16.8 (11.0–23.4)	17 (10.7–26.0)	0.940
Underlying illness			
any disease	18 (40)	15 (27)	0.159
asthma	13 (29)	4 (7)	0.004
allergy	13 (29)	11 (20)	0.278
Influenza vaccination in previous season (2005–2006)	35 (78)	7 (13)	<0.001
Medical office visit within 6 months for cold-like illness	30 (67)	37 (66)	0.950
Past history of hospitalization	12 (27)	25 (27)	0.950
Sleeping hours	10.0 (8.0–11.0)	9.0 (7.0–11.0)	0.012
Influenza vaccination of family members	36 (80)	21 (38)	<0.001
Number of family members	4 (3–8)	4.5 (2–8)	0.075
Number of siblings	2 (1–4)	2 (1–4)	0.337
Room space per person (m^2)	21.7 (7.03–49.5)	14.7 (6.25–47.0)	0.019
Presence of smoker in the family	34 (76)	31 (55)	0.035

Values are expressed as median (range) or number (%), unless otherwise indicated.
[1]Chi-square test or Wilcoxon rank sum test employed where appropriate.

Table 2. Odds ratios of 2006–07 influenza vaccination for outcomes during the entire study period (weeks 1 through 15 of 2007).

	Number (%)		Univariate		Multivariate[1]	
outcomes	Vaccinee (N=45)	Nonvaccinee (N=56)	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Fa	21(47)	37 (66)	0.45 (0.20–1.01)	0.052	1.02 (0.25–4.26)	0.974
FaS	19 (42)	36 (64)	0.41 (0.18–0.91)	0.028	0.291 (0.072–1.143)	0.0762
FaSS	18 (40)	33 (59)	0.47 (0.21–1.03)	0.060	0.289 (0.069–1.142)	0.0761
Fb	16 (36)	23 (41)	0.79 (0.35–1.78)	0.572	0.82 (0.22–2.98)	0.758
FbS	13 (29)	23 (41)	0.58 (0.25–1.35)	0.206	0.285 (0.070–1.141)	0.0759
FbSS	12 (27)	23 (41)	0.52 (0.22–1.23)	0.133	0.286 (0.070–1.140)	0.0758

Note: OR: odds ratio, CI: confidence interval, Fa: fever ≥38.0°C alone, FaS: Fa plus ≥ one respiratory symptoms (rhinorrhea, cough and/or sore throat), FaSS: Fa plus ≥ 2 respiratory symptoms, Fb: ≥39.0°C alone, FbS: Fb plus ≥ one respiratory symptoms, FbSS: Fb plus ≥ 2 respiratory symptoms.
[1]Adjusted for age, sex, influenza vaccination of family members, asthma, sleeping hours, number of family members, presence of smoker in the family, 2005–2006 influenza vaccination, room space per person.

decreased more than with univariate analysis. After adjustment for potential confounders, multivariate analysis showed a 68% ([1-0.16]/[1-0.50]) increase in the efficacy for preventing FbSS as compared to univariate analysis (**Table 5**).

Discussion

The entire period (weeks 1-15 of 2007: January 1-April 14) of this study is generally the epidemic season for influenza in Japan, therefore ILI patients increased among the study subjects from the beginning of the observation period. However, ORs for FaS, FaSS, FbS and FbSS were decreased, but not significantly throughout the entire period. This might have been because the presence of patients having respiratory infection due to non-influenza illnesses might have led to underestimation of the effectiveness of the vaccine.

According to the distribution of ILI patients in the nursery school (**Fig. 1B**), there were patients who were RSV positive from the first week through the fifth week. At the same time, a small RSV epidemic was detected by using regional surveillance information during the period in Fukuoka prefecture. Therefore, it is possible that the outbreak of RSV infection might have overlapped the influenza epidemic in this period (weeks 1-5) among the study subjects. There was no significant reduction in the ORs of any outcome indicators in the RSV infection outbreak period (weeks 1-5: January 1-February 3) among the nursery school children, perhaps because ILI patients among the study subjects might not have had influenza in this period.

In the multivariate analysis, only the OR for FbSS, which was the most severe outcome level, significantly decreased in the local influenza epidemic period (weeks 6-14: February 4-April 7). The following interpretations could explain this result. First, the observation period was limited to the local influenza epidemic period by the use of regional surveillance information. Therefore patients with RSV infection **might have been congregated with** the ILI patients. However, it is highly probable that this would have occurred in any case because this period was immediately after the outbreak of RSV infection (weeks 5–8) among the study subjects. RSV infection might be severe among infants aged < 1 year, but the fever and respiratory symptoms are usually mild.[23] Therefore, adoption of rigorous outcome classification levels could decrease the congregation of patients having RSV infection with ILI patients (**Fig. 1B**). Consequently, the OR for FbSS, the most severe level, might have significantly decreased.

In the analysis during the influenza outbreak among the nursery school children (weeks 10-14: March 4-April 7), multivariate analysis revealed that vaccination was effective at preventing FaS, FaSS, FbS and FbSS. The effectiveness of the vaccine for FbSS, compared with result of the local influenza epidemic period, increased by 5% ([1-0.16])/([1-0.20]). The distribution of ILI patients with detected influenza virus among the study subjects was consistent with this observation period (weeks 8-15). Therefore this period appears to have been the influenza outbreak period among the study subjects. Consequently, the outcome misclassification could be minimized because ILI patients in this period were more likely to have true influenza. However,

Table 3. Odds ratios of 2006–07 influenza vaccination for outcomes during RSV infection epidemic period at nursery school (weeks 1 through 5 of 2007).

	Number (%)		Univariate		Multivariate[1]	
outcomes	Vaccinee (N=45)	Nonvaccinee (N=56)	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Fa	11 (24)	18 (32)	0.68 (0.28–1.65)	0.397	0.65 (0.14–2.91)	0.570
FaS	10 (22)	16 (29)	0.71 (0.29–1.78)	0.469	0.53 (0.11–2.55)	0.432
FaSS	10 (22)	12 (21)	1.05 (0.41–2.71)	0.923	0.82 (0.17–4.05)	0.805
Fb	6 (13)	9 (16)	0.80 (0.26–2.46)	0.701	1.25 (0.20–7.64)	0.813
FbS	5 (11)	6 (16)	0.65 (0.20-2.11)	0.476	0.65 (0.10-4.32)	0.660
FbSS	5 (11)	8 (14)	0.75 (0.23-2.47)	0.637	0.57 (0.08-3.91)	0.566

Note: same as **Table 2**.

Table 4 Odds ratios of 2006-07 influenza vaccination for outcomes during influenza epidemic period in Fukuoka prefecture (weeks 6 through 14 of 2007).

outcomes	Number (%)		Univariate		Multivariate[1]	
	Vaccinee (N=45)	Nonvaccinee (N=56)	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Fa	15 (33)	30 (54)	0.43 (0.19-0.98)	0.044	0.64 (0.17-2.40)	0.504
FaS	13 (29)	30 (54)	0.35 (0.15-0.81)	0.014	0.31 (0.08-1.19)	0.089
FaSS	11 (24)	26 (46)	0.37 (0.16-0.88)	0.025	0.25 (0.06-1.01)	0.051
Fb	12 (27)	18 (32)	0.77 (0.32-1.83)	0.549	0.53 (0.13-2.11)	0.367
FbS	10 (22)	18 (32)	0.60 (0.25-1.48)	0.271	0.22 (0.05-1.00)	0.050
FbSS	8 (18)	17 (30)	0.50 (0.19-1.29)	0.149	0.20 (0.04-0.94)	0.041

Note: same as **Table 2**.

multivariate ORs for Fa and Fb, outcome classifications with only a fever, did not significantly decrease. These results indicated that a fever without respiratory symptoms might not be a symptom of influenza. On the other hand, even if the outcome was fever only, the point estimates in this period were lower than those in other observation periods. The reason for this may have been that the patients who had only a fever were likely to mix with true influenza patients during the peak influenza epidemic period. Moreover, the point estimates gradually decreased in the order Fa, FaS and FaSS or Fb, FbS and FbSS in the multivariate analysis, as with the results for the local influenza epidemic period. In addition, their 95%CIs gradually became narrower. These results indicated that following procedures such as determining the peak of the influenza epidemic, adopting a rigorous outcome classification system and adjusting potential confounders could minimize outcome misclassification.

The number of patients affected by RSV, which causes lower respiratory tract infection, was comparatively small, even in the RSV epidemic period. However, numerous outbreaks of RSV infection are reported among communal populations such as families, hospitalized children and elderly people in nursing homes. Thus, it is possible that RSV infection is masked by influenza infection in many patients. Therefore, it might be difficult to identify outbreaks of RSV infection among certain populations by using only regional surveillance information.

In this study, we used information about not only influenza but also RSV infection obtained from regional surveillance to identify the outbreak of influenza among the study subjects. Additionally, pathogen detection was performed using rapid diagnostic tests for some of the subjects. Therefore, we were able to detect the outbreak of RSV infection in the nursery school. Consequently, the main observation could be limited to the peak influenza epidemic period. Furthermore, adoption of the rigorous case classification system made it possible to minimize outcome misclassification of patients with RSV infection as influenza patients, even if the influenza epidemic overlapped the circulation of RSV.

In our study, we found that the effectiveness of the vaccine was higher than that reported in a previous study that evaluated inactivated influenza vaccine among young children.[24] In the previous study, since the subjects were recruited from several different areas of Japan, the definition of the peak epidemic period of influenza might not have been optimal. Considering our results, if the observations of the previous study were limited to the optimal peak epidemic period of influenza among the study subjects, the effectiveness of the vaccine might have been found to be higher. However, on a critical review and re-analysis of 15 meta-data, parenteral inactivated influenza vaccine efficacy or effectiveness against children remains scarce.[25] Therefore, standard setting about various points as follows may be required.

Influenza epidemics follow different patterns depending on the time, place, and population.[11] According to this principle, analysis in the same epidemic season, in the same area and among a homogeneous population for exposure to influenza could be the key to evaluating the effectiveness influenza vaccine correctly. Moreover, the following procedures are essential to minimize the effect of outcome misclassification in field trials of influenza vaccine effectiveness.[26-28] First, all study subjects should be followed

Table 5 Odds ratios of 2006-07 influenza vaccination for each outcome during influenza outbreak period at the nursery school (weeks 10 through 14 of 2007).

outcomes	Number (%)		Univariate		Multivariate[1]	
	Vaccinee (N=45)	Nonvaccinee (N=56)	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Fa	13 (29)	29 (52)	0.38 (0.17-0.87)	0.022	0.39 (0.11-1.42)	0.152
FaS	12 (27)	28 (50)	0.36 (0.16-0.85)	0.019	0.23 (0.06-0.91)	0.036
FaSS	10 (22)	25 (45)	0.35 (0.15-0.85)	0.021	0.21 (0.05-0.85)	0.029
Fb	10 (22)	16 (29)	0.71 (0.29-1.78)	0.470	0.32 (0.07-1.48)	0.145
FbS	9 (20)	16 (29)	0.63 (0.25-1.59)	0.323	0.18 (0.04-0.93)	0.041
FbSS	7 (16)	15 (27)	0.50 (0.19-1.37)	0.179	0.16 (0.03-0.87)	0.033

Note: same as **Table 2**.

with equal intensity. Second, the influenza epidemic should be relatively large. Third, the circulating influenza viruses should antigenically match the vaccine strains. In this study, parents or guardians collected information on the children's body temperatures and symptoms each week during the entire follow-up period using a questionnaire, so all subjects were followed with almost equal intensity. In addition, all the subjects were recruited from a single nursery school, so their exposure to influenza might have been homogeneous. There was a relatively large epidemic of influenza that exceeded 60 patients per sentinel hospital in the peak epidemic period in Fukuoka prefecture during the 2006-07 influenza season. Furthermore type A H3N2 and type B were mainly cocirculatiing during this season, and these strains matched the vaccine strains.

These results suggested that confining observation to the peak influenza epidemic period and adoption of rigorous case definitions were both essential techniques to minimize outcome misclassification for analysis of the effectiveness of influenza vaccine against ILI under these advantageous conditions in for a field trial of influenza vaccine.

We detected high effectiveness of the influenza vaccine among nursery school children during the epidemic season when influenza cocirculated with RSV infection, which is difficult to distinguish from influenza. We

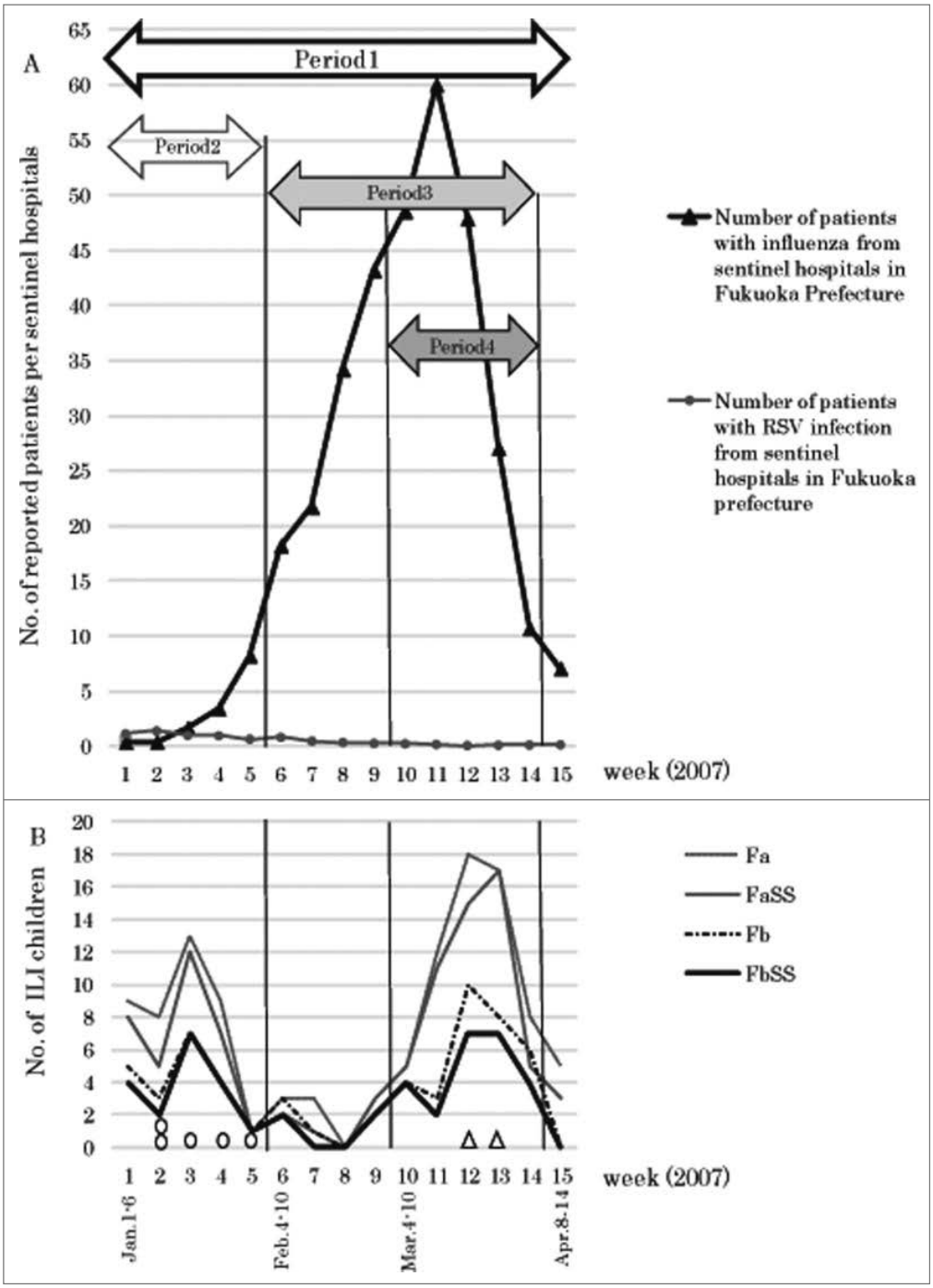

Figure 1. (**A**) Numbers of patients with influenza and RSV infections reported weekly from sentinel hospitals in Fukuoka Prefecture. (**B**) The cumulative total numbers of patients among the nursery school children classified by the case definitions (Fa: ≥38.0°C alone, FaSS: Fa plus ≥2 respiratory symptoms, Fb: ≥ 39.0°C alone FbSS: Fb plus ≥2 respiratory symptoms). Positive cases confirmed by rapid diagnostic tests for influenza virus and RSV among patients ≥37.5°C are shown as influenza virus:△ and RSV:○. Four observational periods were defined as follows: period 1, from weeks 1 through 15 of 2007 (the entire observation period); period 2, from weeks 1 through 5 (the period of the outbreak of RSV infection among the nursery school children); period 3, from weeks 6 through 14 (the period of the influenza epidemic in Fukuoka Prefecture); and period 4, from weeks 10 through 14 (the period of the outbreak of influenza among the nursery school children).

succeeded in minimizing outcome misclassification by using special techniques to identify the peak of the influenza epidemic and by adopting a rigorous case classification system. These methods can generally be applied to evaluation of the efficacy of the influenza vaccine for ILI.

When ILI is used as the study outcome, it is less specific for influenza than laboratory confirmation. Nevertheless, as an outcome indicator it is more useful in actual field trials. Laboratory confirmation is normally expensive, especially in developing countries. In this study, we used both information obtained from regional surveillance and the distribution of patients in the nursery school. Detection of pathogens was conducted by using rapid diagnostic tests for influenza and RSV among some patients. Furthermore, adoption of a rigorous case classification system enabled us to differentiate patients with RSV infection from those with ILI patients during the influenza epidemic. Consequently, the effectiveness of the vaccine during the influenza outbreak in the nursery school approximated that among patients with true influenza.

Limitations

The sample size was small in this study. However, the point estimates for all outcomes during the period of the influenza epidemic were consistently less than 1, with a narrow 95% CI. Therefore, we considered that the reliability of results was maintained. The effects of potential confounders were taken into consideration in multivariate analysis in this study. Variables associated with potential confounders besides the age in months and gender among the young children were included in the model for adjustment. However, the possibility of residual confounders cannot be denied. In addition, the disease investigation among study subjects was conducted by their parents and guardians. This enabled us to follow all the study subjects with equal intensity, but disease misclassification might have occurred. However, this is a nondifferential misclassification. If there were such a misclassification, it would be underestimated in the results.

Materials and methods

Study subjects

The study subjects were 111 children (45 who were vaccinated twice, 10 who were vaccinated once, and 56 who had not been vaccinated) aged 12 to 84 months, recruited from Shin Yoshitomi nursery school, Koge-machi, Fukuoka Prefecture. In Japan, vaccination of seasonal inactivated influenza vaccine has been recommended 2 doses from children aged between 6 months to 12 years due to the relatively weak immune response toward the vaccine. Among 111 subjects, 10 children fail to have 2-dose vaccination. For the critical analysis of the influenza vaccine efficacy, we focused on the 2-dose population. Thus, one-dose subjects (10 children), were excluded from the final sample. Finally, the analysis subjects comprised 101 children. The twice-vaccinated subjects had received commercially obtained 2006-07 trivalent inactivated influenza vaccine with an interval of at least 2 weeks between the 2 vaccinations in each clinic during November 4 to December 24, 2006. Each vaccination was given at the then recommended dose (0.2 mL for children aged 12 months to <72 months and 0.3 mL for age ≥72 months). The vaccine contained A/ New Caledonia/ 20/ 99 (H1N1), A/ Hiroshima/ 52/ 2005 (H3N2) and B/ Malaysia/ 2506/ 2004, 30 μg of hemagglutinin per 1 mL from each strain. Informed consent was obtained from the parent or guardian of each subject. The study was conducted with the approval of the Ethics Committee of the Graduate School of Medicine, Osaka City University.

Information collection

At the time of enrollment, the following information was collected as continuous data by means of a self-administered questionnaire given to each child's parent or guardian: gender, age in months, body weight, hours of sleep per day, number of family members, number of siblings and floor area of residence. As categorical data, underlying disease, the history of influenza vaccination in the preceding season, history of medical examination for cold-like symptoms during the previous 6 months, history of hospitalization, influenza vaccination of family members (in the 2006-07 season) and smoking by family members was ascertained.

As a follow-up survey, information about the following was collected by means of a weekly self-administered questionnaire from each child's parent or guardian: fever, cough, rhinorrhea, sore throat, joint pain and chills. The follow-up period was January 1 to April 14, 2007 (15 weeks). This information was submitted every week to the nursery school by the children's parents or guardians.

The epidemic in Fukuoka prefecture and the outbreaks in the nursery school of influenza and RSV infection.

Influenza epidemics normally occur from early January to mid-April in Japan. ILI patients were found at the nursery school from the first week (Jan. 1–6) that the observation started. At the time of starting observation, a small outbreak of RSV infection was confirmed at the same time as an influenza epidemic by the regional surveillance system in Fukuoka Prefecture. Therefore, for viral surveillance of ILI patients at the nursery school, when a subject developed temperature of ≥37.5°C, the school nurse collected nasal discharge from the subject.

Pathogen detection from collected samples was conducted using rapid diagnostic tests for influenza virus, RSV and adenovirus at the Osaka Prefectural Institute of Public Health. In Fukuoka prefecture, the number of patients with RSV infection reported weekly by the sentinel hospitals (**Fig. 1A**) reached a peak in the second week of 2007 (1.4 patients) and then gradually declined until week 15. On the other hand, the number of influenza patients exceeded 10 patients weekly as reported by the sentinel hospitals from the sixth week. The peak number was found in week 11 (60.1 patients). Thereafter, the number of influenza patients decreased rapidly, becoming less than 10 patients per sentinel hospital in week 15. The weekly occurrence of ILI patients at the nursery school is illustrated in **Figure 1B**. There were 2 peaks of the occurrence of ILI patients, in week 3 and week 13, approximately corresponding to a small peak of RSV infection and the peak of the influenza, respectively, in Fukuoka prefecture. Additionally, in the nursery school children, RSV was detected in 5 patients from the second through the fifth

weeks and influenza virus was detected in 2 patients in weeks 12 and 13 by rapid diagnostic tests (**Fig. 1B**).

Analysis

We classified the cases into 6 levels of ILI for outcomes: (1) Fa: fever ≥ 38.0°C alone, (2) FaS: Fa plus ≥ one respiratory symptoms (rhinorrhea, cough and/or sore throat), (3) FaSS: Fa plus ≥ 2 respiratory symptoms, (4) Fb: fever ≥ 39.0°C alone, (5) FbS: Fb plus ≥ one respiratory symptoms, (6) FbSS: Fb plus ≥ 2 respiratory symptoms.

Four observation periods were set up (**Fig. 1**). (1) The entire observation period (weeks 1-15 of 2007: January 1-April 14), (2) The RSV infection outbreak period among the nursery school children: the period during which there were ≥5 FaSS patients and RSV was detected from these patients by using a rapid diagnostic test at the nursery school (weeks 1-5: January 1-February 3), (3) the local influenza epidemic period during which ≥10 influenza patients were reported weekly by the sentinel hospitals in Fukuoka Prefecture (weeks 6-14: February 4-April 7), and (4) the influenza outbreak period among the nursery school children, during which there were ≥5 FaSS patients and influenza virus was detected in these patients by using a rapid diagnostic test at the nursery school (weeks 10-14: March 4-April 7). Furthermore, the total cumulative number of FaSS patients was assessed each week.

To compare the characteristics of vaccinees and nonvaccinees, the chi-square test or Fisher's exact test, and the Wilcoxon rank-sum test were employed. Logistic regression models were used to calculate the odds ratios (OR) and 95% confidence interval (CI) of vaccination for outcome indicators 1 to 6. In multivariate analysis, age in months and gender were put into the model as variables, and other variables that were different between vaccinees and nonvaccinees with *P* values of less than 0.1 were added to the model. All reported *P* values were 2-sided values of 5%. All data analyses were carried out using SAS Version 9.3 (SAS Institute, Inc, Cary, North Carolina).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Poehling KA, Edwards KM, Weinberg GA, Szilagyi P, Staat MA, Iwane MK, Bridges CB, Grijalva CG, Zhu Y, Bernstein DI, et al. The underrecognized burden of influenza in young children. N Engl J Med 2006; 355:31-40; PMID:16822994; http://dx.doi.org/10.1056/NEJMoa054869
2. Fu C, He Q, Li Z, Xu J, Li Y, Lu J, Li K, Yang Q, Dong Z, Liu X, et al. Seasonal influenza vaccine effectiveness among children, 2010-2012. Influenza Other Respi Viruses 2013; 7(6):1168-74; PMID:23981250
3. Shen S, Campitelli MA, Calzavara A, Guttmann A, Kwong JC. Seasonal influenza vaccine effectiveness in pre- and full-term children aged 6-23 months over multiple seasons. Vaccine 2013; 31:2974-8; PMID:23688524; http://dx.doi.org/10.1016/j.vaccine.2013.05.011
4. Staat MA, Griffin MR, Donauer S, Edwards KM, Szilagyi PG, Weinberg GA, Hall CB, Prill MM, Chaves SS, Bridges CB, et al. Vaccine effectiveness for laboratory-confirmed influenza in children 6-59 months of age, 2005-2007. Vaccine 2011; 29:9005-11; PMID:21945256; http://dx.doi.org/10.1016/j.vaccine.2011.09.037
5. Eisenberg KW, Szilagyi PG, Fairbrother G, Griffin MR, Staat M, Shone LP, Weinberg GA, Hall CB, Poehling KA, Edwards KM, et al. Vaccine effectiveness against laboratory-confirmed influenza in children 6 to 59 months of age during the 2003-2004 and 2004-2005 influenza seasons. Pediatrics 2008; 122:911-9; PMID:18977968; http://dx.doi.org/10.1542/peds.2007-3304
6. Allison MA, Daley MF, Crane LA, Barrow J, Beaty BL, Allred N, Berman S, Kempe A. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003-2004 season. J Pediatr 2006; 149:755-62; PMID:17137887; http://dx.doi.org/10.1016/j.jpeds.2006.06.036
7. Fujieda M, Maeda A, Kondo K, Kaji M, Hirota Y. Inactivated influenza vaccine effectiveness in children under 6 years of age during the 2002-2003 season. Vaccine 2006; 24:957-63; PMID:16171903; http://dx.doi.org/10.1016/j.vaccine.2005.08.083
8. Shuler CM, Iwamoto M, Bridges CB, Marin M, Neeman R, Gargiullo P, Yoder TA, Keyserling HL, Terebuh PD. Vaccine effectiveness against medically attended, laboratory-confirmed influenza among children aged 6 to 59 months, 2003-2004. Pediatrics 2007; 119:E587-E95; PMID:17332179; http://dx.doi.org/10.1542/peds.2006-1878
9. Hurwitz ES, Haber M, Chang A, Shope T, Teo ST, Giesick JS, Ginsberg MM, Cox NJ. Studies of the 1996-1997 inactivated influenza vaccine among children attending day care: immunologic response, protection against infection, and clinical effectiveness. J Infect Dis 2000; 182:1218-21; PMID:10979921; http://dx.doi.org/10.1086/315820
10. Vesikari T, Fleming DM, Aristegui JF, Vertruyen A, Ashkenazi S, Rappaport R, Skinner J, Saville MK, Gruber WC, Forrest BD. Safety, efficacy, and effectiveness of cold-adapted influenza vaccine-trivalent against community-acquired, culture-confirmed influenza in young children attending day care. Pediatrics 2006; 118:2298-312; PMID:17142512; http://dx.doi.org/10.1542/peds.2006-0725
11. Monto AS. Occurrence of respiratory virus: time, place and person. Pediatr Infect Dis J 2004; 23:S58-64; PMID:14730271; http://dx.doi.org/10.1097/01.inf.0000108193.91607.34
12. Anderson LJ, Parker RA, Strikas RL. Association between respiratory syncytial virus outbreaks and lower respiratory tract deaths of infants and young children. J Infect Dis 1990; 161:640-6; PMID:2319164; http://dx.doi.org/10.1093/infdis/161.4.640
13. Sugaya N, Mitamura K, Nirasawa M, Takahashi K. The impact of winter epidemics of influenza and respiratory syncytial virus on paediatric admissions to an urban general hospital. J Med Virol 2000; 60:102-6; PMID:10568771; http://dx.doi.org/10.1002/(SICI)1096-9071(200001)60:1%3c102::AID-JMV17%3e3.0.CO;2-D
14. Kaneko M, Watanabe J, Kuwahara M, Ueno E, Hida M, Kinoshita A, Sone T. Impact of respiratory syncytial virus infection as a cause of lower respiratory tract infection in children younger than 3 years of age in Japan. J Infect 2002; 44:240-3; PMID:12099731; http://dx.doi.org/10.1053/jinf.2002.0981
15. Hall CB, Geiman JM, Biggar R, Kotok DI, Hogan PM, Douglas GR. Respiratory syncytial virus infections within families. N Engl J Med 1976; 294:414-9; PMID:173995; http://dx.doi.org/10.1056/NEJM197602192940803
16. Aymard M, Chomel JJ, Allard JP, Thouvenot D, Honegger D, Floret D, Boissel JP, Collet JP, Dürr F, Gillet J, et al. Epidemiology of viral infections and evaluation of the potential benefit of OM-85 BV on the virologic status of children attending day-care centers. Respiration 1994; 61 Suppl 1:24-31; PMID:7800968; http://dx.doi.org/10.1159/000196377
17. Harrington RD, Hooton TM, Hackman RC, Storch GA, Osborne B, Gleaves CA, Benson A, Meyers JD. An outbreak of respiratory syncytial virus in a bone marrow transplant center. J Infect Dis 1992; 165:987-93; PMID:1583345; http://dx.doi.org/10.1093/infdis/165.6.987
18. Kohno H. [Observation over three seasons of respiratory syncytial virus infection in a geriatric health service facility]. Nihon Ronen Igakkai Zasshi 2012; 49:608-11. Japanese; PMID:23459652; http://dx.doi.org/10.3143/geriatrics.49.608
19. Mizuta K, Abiko C, Aoki Y, Ikeda T, Matsuzaki Y, Itagaki T, Katsushima F, Katsushima Y, Noda M, Kimura H, Ahiko T. Seasonal patterns of respiratory syncytial virus, influenza A virus, human metapneumovirus, and parainfluenza virus type 3 infections on the basis of virus isolation data between 2004 and 2011 in Yamagata, Japan. Jpn J Infect Dis 2013; 66:140–5.

20. de-Paris F, Beck C, Machado AB, Paiva RM, da Silva Menezes D, de Souza Nunes L, Kuchenbecker R, Barth AL. Optimization of one-step duplex real-time RT-PCR for detection of influenza and respiratory syncytial virus in nasopharyngeal aspirates. J Virol Methods 2012; 186:189-92; PMID:22796284; http://dx.doi.org/10.1016/j.jviromet.2012.07.008
21. Karadag-Oncel E, Ciblak MA, Ozsurekci Y, Badur S, Ceyhan M. Viral etiology of influenza-like illnesses during the influenza season between December 2011 and April 2012. J Med Virol 2014; 86: 865-71; PMID:24105787; http://dx.doi.org/10.1002/jmv.23747
22. Zimmerman RK, Rinaldo CR, Nowalk MP, Gk B, Thompson MG, Moehling KK, Bullotta A, Wisniewski S. Influenza and other respiratory virus infections in outpatients with medically attended acute respiratory infection during the 2011-12 influenza season. Influenza Other Respir Viruses 2014; 8: 397-405; PMID:24852890; http://dx.doi.org/10.1111/irv.12247
23. Borchers AT, Chang C, Gershwin ME, Gershwin LJ. Respiratory syncytial virus–a comprehensive review. Clin Rev Allergy Immunol 2013; 45:331-79; PMID:23575961; http://dx.doi.org/10.1007/s12016-013-8368-9
24. Ochiai H, Fujieda M, Ohfuji S, Fukushima W, Kondo K, Maeda A, Nakano T, Kamiya H, Hirota Y; Influenza Vaccine Epidemiology Study Group. Inactivated influenza vaccine effectiveness against influenza-like illness among young children in Japan—with special reference to minimizing outcome misclassification. Vaccine 2009; 27:7031-5; PMID:19786133; http://dx.doi.org/10.1016/j.vaccine.2009.09.067
25. Manzoli L, Ioannidis JP, Flacco M, De Vito C, Villari P. Effectiveness and harms of seasonal and pandemic influenza vaccines in children, adults and elderly: a critical review and re-analysis of 15 meta-analyses. Hum Vaccin Immunother 2012; 8: 851-62; PMID:22777099; http://dx.doi.org/10.4161/hv.19917
26. Hirota Y, Kaji M. [Principles and methods of influenza epidemiology: with special reference to field evaluation of vaccine efficacy]. Kansenshogaku Zasshi 1994; 68:1293-305. Japanese; PMID:7829897; http://dx.doi.org/10.11150/kansenshogakuzasshi1970.68.1293
27. Hirota Y, Kaji M, Ide S, Kajiwara J, Kataoka K, Goto S, Oka T. Antibody efficacy as a keen index to evaluate influenza vaccine effectiveness. Vaccine 1997; 15:962-7; PMID:9261942; http://dx.doi.org/10.1016/S0264-410X(96)00302-7
28. Ozasa K. The effect of misclassification on evaluating the effectiveness of influenza vaccines. Vaccine 2008; 26:6462-5; PMID:18573297; http://dx.doi.org/10.1016/j.vaccine.2008.06.039

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Effectiveness of acellular pertussis vaccine in a routine immunization program: A multicenter, case-control study in Japan

Satoko Ohfuji[a,*], Kenji Okada[b], Takashi Nakano[c], Hiroaki Ito[d], Megumi Hara[e], Haruo Kuroki[f], Yoshio Hirota[a,g]

[a] Department of Public Health, Osaka City University Faculty of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan
[b] Section of Pediatrics, Department of Medicine, Fukuoka Dental College, 2-15-1, Tamura, Sawara-ku, Fukuoka 814-0193, Japan
[c] Department of Pediatrics, Kawasaki Medical School, 577, Matsushima, Kurashiki, Okayama 701-0192, Japan
[d] Field Epidemiology Training Program, National Institute of Infectious Diseases, 1-23-1, Toyama, Shinjuku-ku, Tokyo 162-8640, Japan
[e] Department of Preventive Medicine, Faculty of Medicine, Saga University, 5-1-1, Nabeshima, Saga 849-8501, Japan
[f] Sotobo Children's Clinic, Medical Corporation Shigyo-no-kai, 1880-4, Izumi, Misaki-cho, Isumi 299-4503, Chiba, Japan
[g] Clinical Epidemiology Research Center, Medical Co. LTA, 6-18, Ten-ya-machi, Hakata-ku, Fukuoka 812-0025, Japan

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ABSTRACT

In 2008, the number of pertussis cases increased substantially among Japanese adolescents, despite high coverage with acellular pertussis vaccine (DTaP). This study examined the effectiveness of DTaP vaccine in the routine immunization program in Japan. Between April 2009 and October 2012, we conducted a multicenter, case-control study, and compared the history of DTaP vaccination between 55 newly diagnosed pertussis cases and 90 age- and sex-matched controls. DTaP vaccine history was obtained by a self-administered questionnaire completed by their parents or guardians. Logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) of vaccination for development of pertussis.

DTaP vaccination of ≥1 dose revealed a significantly lower OR for pertussis (OR = 0.20, 95%CI, 0.04–0.97), and the OR of complete vaccination (4 doses) was 0.22 (0.04–1.05). Even after limiting subjects to those whose vaccination status could be confirmed by the immunization records, the negative associations were observed. The decreasing ORs of 4-dose vaccinees remained, even among subjects who had received the fourth dose ≥9.2 years earlier (OR = 0.11, 95%CI, 0.01–1.02).

In conclusion, DTaP vaccination had a preventive effect for pertussis. Effectiveness was observed even 9 or more years after the final dose.

1. Introduction

In Japan, the routine immunization program with pertussis vaccine was temporarily suspended in 1975 due to concern about severe adverse events such as encephalopathy [1–4]. Two months later, the immunization program was resumed, but vaccine coverage had been extremely low until acellular pertussis vaccine combined with diphtheria–tetanus toxoids (DTaP vaccines) was introduced for children over 24 months in late 1981. Afterward, the age of administration of DTaP vaccine was changed to 3 months in 1988, and vaccine coverage improved to about 90% in the late 1990s. Through these strategies, the annual number of reported pertussis cases decreased to about 10,000 in the early 2000s [3]. However, despite high vaccination coverage (i.e., over 90% in every year), the number of reported pertussis cases increased in the late 2000s. According to the age distribution of reported pertussis cases, the proportion of adolescents and adults has been increasing, and the proportion reached half in 2008 [5]. The reason why pertussis cases have been increasing among adolescents and adults is not completely clear. However, several reasons, such as improved diagnostics, the lower vaccine coverage era between 1975 and 1981, or waning immunity among those who had received DTaP vaccination in childhood, may be responsible [6,7].

Based on the present Japanese immunization program with DTaP vaccination, children receive 4 doses of DTaP, including 3 primary doses at the ages of 3, 4, and 5 months, and 1 booster

Abbreviations: DTaP vaccines, acellular pertussis vaccine combined with diphtheria–tetanus toxoids; LAMP method, loop-mediated isothermal amplification method; PT-IgG, IgG antibody for pertussis toxin; OR, odds ratio; CI, confidence interval.

* Corresponding author. Tel.: +81 6 6645 3756; fax: +81 6 6645 3757.
E-mail address: satop@med.osaka-cu.ac.jp (S. Ohfuji).

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dose at 18 to 23 months. On the other hand, in the United States of America, the Advisory Committee on Immunization Practices recommends 5 doses of DTaP vaccination for childhood (2, 4, 6, 15 to 18 months, and 4 to 6 years of age) and an adolescent booster dose of the tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (Tdap) at 11 to 12 years [8]. According to previous studies, the clinical effectiveness of DTaP vaccine for pertussis has been weakening with time after the final dose of pertussis vaccine [9–13], which suggests that an adolescent booster dose of vaccination might also be needed in Japan.

Thus, a hospital-based case-control study was conducted to examine the effectiveness of DTaP vaccine in preventing the development of pertussis in the present routine immunization program. The present study also evaluated the effect of 4-dose vaccination for pertussis separately by time since the fourth dose of DTaP.

2. Materials and methods

2.1. Selection of cases and controls

Between April 2009 and October 2012 (the study period), a multicenter, case-control study was conducted in Japan. Newly diagnosed cases of pertussis were recruited at 4 collaborating hospitals in 4 different areas of Japan: (from north to south) Chiba, Saitama, Mie, and Fukuoka. Eligible cases were newly diagnosed pertussis patients who satisfied the clinical criteria for pertussis and whose age at diagnosis was less than 30 years. The clinical criteria for pertussis were: cough lasting for more than 7 days with one or more symptoms (paroxysmal cough, whoop, or posttussive vomiting) and one of isolation of *Bordetella pertussis*, positive results by the loop-mediated isothermal amplification (LAMP) method, serodiagnosis (for paired serum samples at the acute phase and at the recovery phase, at least twofold increase of IgG antibody for pertussis toxin (PT-IgG) or fourfold increase of agglutinin titer, while for a single serum sample at the acute phase, PT-IgG of 10 EU/mL or more among unvaccinated subjects or 100 EU/mL or more among vaccinated subjects) or epidemiologically linked to a confirmed pertussis case. During the study period, a pertussis outbreak occurred in Saga University [14], where one of the investigators worked. Thus, if cases diagnosed in Saga University satisfied the clinical criteria in the present study, they also contributed to the present study.

Regarding the recruitment of control subjects, each case was asked to provide up to five friend controls, of the same age (or school grade) and sex as the case. Exclusion criteria were: presence of lasting cough for more than 1 week during the 1 month prior to case diagnosis. During the study period, however, it turned out that some cases (particularly preschool children) did not have any friends and could not provide any friend controls. Thus, not only friend controls but also hospital controls were recruited for cases who were enrolled since April 2012. Collaborating hospitals were encouraged to select up to five hospital controls among patients without pertussis, matching for age and sex.

The study protocol was approved by the ethics committees at the Osaka City University Faculty of Medicine and at the collaborating hospitals, and written, informed consent was obtained from all subjects (or their parents or guardians) prior to participation.

2.2. Information collection

The following information was obtained by means of a self-administered questionnaire completed by each child's parent or guardian: sex, date of birth; history of pertussis; history of DTaP vaccination, number of vaccinations, vaccination dates, vaccine manufacturer and vaccine lot number if vaccinated; underlying illnesses (heart disease, renal disease, liver disease, diabetes mellitus, anemia, asthma, other respiratory diseases, tonsillitis, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, immunodeficiency, epilepsy); history of steroid treatment for more than one month; total room space in the house (m^2); number of family members; contact with a confirmed pertussis case during the recent one month; and contact with a person with a lasting cough during the last month. In Japan, the vaccination history is usually recorded in an immunization record book maintained by individuals. Thus, the information collected about vaccination status was confirmed by the immunization record. When missing answers or illogical data were detected by research technicians, research technicians conducted a telephone interview to complete the data.

In addition, for pertussis cases, the following clinical findings were reported by the pediatricians-in-charge using a standardized questionnaire: date at symptom onset; date at diagnosis; disease symptoms (paroxysmal cough, whoop, posttussive vomiting, fever, dyspnea, and seizures); and laboratory examinations (culture isolation of *B. pertussis* and results by the LAMP method, and PT-IgG and agglutinin titers in the acute and recovery phases).

2.3. Statistical analysis

First, it was verified that the background characteristics of hospital controls were not different from those of friend controls using the chi-square test, Fisher's exact test, or the Wilcoxon rank sum test. Then, the characteristics were compared between cases and controls using the chi-square test or the Wilcoxon rank-sum test. Because some cases had no corresponding pair as controls and vice versa, not only a conditional logistic regression model but also an unconditional logistic regression model was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) for pertussis. Trends for associations were assessed by assigning ordinal scores to the level of the independent variable. Variables that showed a *P*-value of less than 0.1 or that seemed to be medically related to the disease were considered potential confounders for adjustment. When unconditional logistic regression models were used, data for not only matched pairs but also unmatched pairs were analyzed, and matching variables (age and sex) were included in the models. Vaccine effectiveness (VE) was calculated as $(1 - \mathrm{OR}) \times 100$ (%).

In addition, to examine the associations between pertussis and 4 doses of DTaP vaccination according to time since the fourth dose, additional analyses were conducted. Time since the fourth dose was calculated as the number of years from the date of the fourth dose to the date of case illness onset or the date of control recruitment. In the analysis, nonvaccinees and 4-dose vaccinees were included, and 4-dose vaccinees were categorized into two or three levels according to the distribution of time since the fourth dose among controls, with the category boundaries chosen so as to make the sizes of the groups as similar as possible. The analysis used unconditional logistic regression models.

All tests were two-sided. All analyses were performed using SAS version 9.1.3 software (SAS Institute, Cary, NC, USA).

3. Results

The sample size required to achieve statistically significant VE was calculated using the power calculation for case-control studies. The calculation was conducted assuming an α level of 0.05, a β level of 0.20, DTaP vaccination proportion in controls of 90%, and OR of vaccine of 0.20. As a result, to achieve statistically significant VE, a total sample size of 90 (30 cases and 60 controls) was needed.

Among the 72 pertussis cases and 97 controls (75 friend controls and 22 hospital controls) enrolled, 63 cases and 94 controls (73 friend controls and 21 hospital controls) responded to the questionnaire (response rate, 88% for cases and 97% for controls). However,

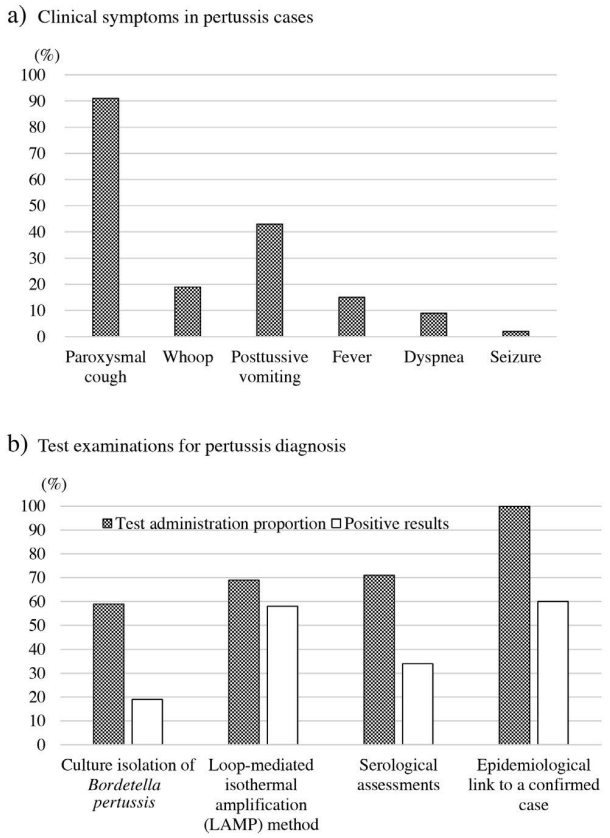

Fig. 1. *Clinical findings in pertussis cases:* (a) clinical symptoms in pertussis cases. (b) Test examinations for pertussis diagnosis.

2 friend controls were subsequently found to be ineligible because they had a history of pertussis. A further 8 cases and 4 controls had incomplete data for the variables and were thus excluded. Eventually, 55 cases and 90 controls (69 friend controls and 21 hospital controls) comprised the subjects for the analysis. Of these, 33 cases and 68 controls (56 friend controls and 12 hospital controls) maintained the matching conditions and were included in the analyses using conditional logistic regression models.

Fig. 1 shows the clinical characteristics of the pertussis cases. About 90% of cases had paroxysmal cough, whereas 19% had an inspiratory whoop and 43% had posttussive vomiting. Among the other symptoms, the proportion having fever was relatively higher (15%) than the others. The median duration from symptom onset to diagnosis was 13.0 days (range: 0–39 days). As for laboratory examinations, culture isolation tests were performed in 60% of cases, and among those, one-third had positive results. LAMP methods were used in 70% of cases, of which 84% had positive results. Among cases who underwent serological assessment (71%), about half had positive results. The number of laboratory-confirmed cases (i.e., positive results for culture isolation, LAMP methods, or serological assessment) was 39 (71%). Based on the information from the self-administered questionnaires, 60% of cases reported contact with a confirmed pertussis case during the last month, which suggested epidemiological linkage to confirmed pertussis cases.

Table 1 shows the background characteristics of the 69 friend controls and 21 hospital controls. A history of steroid treatment seemed to be more common and room space in the house seemed to be larger in hospital controls than in friend controls. Although the small sample size might contribute to the lack of significant differences, further analyses were conducted in all 55 cases and 90 controls, including both friend controls and hospital controls.

The comparison of background characteristics between the 55 cases and 90 controls is shown in Table 2. Among the cases, 12 (22%) were adolescents (i.e., age 11–19 years) and 8 (15%) were adults (i.e., age $\geq$ 20 years). Regarding a history of DTaP vaccination, cases were less likely to have received DTaP vaccine than controls. In addition, cases had more underlying illnesses, more history of steroid treatment, smaller room space in the house, and more contact with a person with a lasting cough.

After adjustment for potential confounders, the OR of DTaP vaccination for development of pertussis was significantly lower in the analysis using unconditional logistic regression models (OR = 0.20, 95%CI, 0.04–0.97) (Table 3). Vaccine effectiveness was calculated to be 80% (3–96%). When considering the effect by the number of DTaP vaccinations, lower ORs were also observed not only in those with complete vaccination (4 doses), but also in those with incomplete vaccination (1–3 doses), with marginal significance. Vaccine effectiveness in those with incomplete vaccination (1–3 doses) was calculated to be 85% (−24 to 98%), and the effectiveness of complete vaccination (4 doses) was 78% (−5 to 96%). In the conditional logistic regression model (33 cases and 68 controls), the magnitudes of the ORs of vaccination were similar to those in the unconditional model, although the limited number of subjects brought about wider confidence intervals (OR of DTaP vaccination = 0.15, 95%CI, 0.01–1.80). On the other hand, a history of steroid treatment and recent contact with a person with a lasting cough showed significantly increased ORs for development of pertussis, using both the unconditional model and the conditional model. In addition, larger room space in the house showed a lower OR for pertussis.

To confirm the association between DTaP vaccination and pertussis, several sensitivity analyses were conducted using unconditional logistic regression models (Table 4). When analyzed subjects were limited to those whose vaccination status could be confirmed by their immunization records, the results were almost unchanged, since 96% of the subjects had their immunization records. In addition, when the analyzed subjects were limited to those aged less than 18 years, since subjects who enrolled from Saga University might have had a different situation on recruitment, similar ORs of DTaP vaccination were observed. When cases were limited to the laboratory confirmed cases, the ORs were almost unchanged, but the confidence intervals were wider. Even when excluding hospital controls from the analysis and comparing 55 cases with 69 friend controls, decreasing ORs were observed.

Table 5 shows the association between pertussis and 4 doses of DTaP vaccination, according to the time since the fourth dose. Unexpectedly, a decreasing OR of DTaP vaccination was observed even among subjects with a longer time since the fourth dose. ORs of 4-dose vaccinees who received the fourth dose within less than 5.8 years, 5.8–9.1 years, and 9.2 years or more were 0.24 (0.05–1.23), 0.14 (0.02–0.87), and 0.11 (0.01–1.02), respectively, all of which were marginally significant.

4. Discussion

In the present case-control study, DTaP vaccine showed effectiveness for preventing the development of pertussis. Although the limited number of study subjects and high vaccination rate in the study subjects made it difficult to detect significant vaccine effectiveness, the present results seemed to support the usefulness of DTaP vaccine in the routine immunization programs.

To date, there have been several studies on pertussis vaccine effectiveness from Japan [14–16]. One study, which was conducted in household contacts when the present immunization programs were introduced (1981–1983), indicated that DTaP vaccine had 79%

Table 1
Comparison of background characteristics between friend-controls and hospital-controls.

Variable		Friend-controls ($n=69$)	Hospital-controls ($n=21$)	P value[b]
		n (%)[a]	n (%)[a]	
Age (years)	Median (range)	10.3 (0.5–25.1)	8.7 (0.3–12.8)	0.142
Sex	Male	23 (33)	7 (33)	1.000
Number of DTaP vaccinations	0	3 (4)	0 (0)	0.486
	1–3	2 (3)	4 (19)	
	4	64 (93)	17 (81)	
Underlying illnesses	Present	15 (22)	6 (29)	0.561
History of steroid treatment	Present	3 (4)	2 (10)	0.331
Total room space in the house (m^2)	Median (range)	102.0 (25–839)	143.0 (25–285)	0.082
Number of family members	Median (range)	4.0 (1–7)	4.0 (3–7)	0.610
Room space per person (m^2)	Median (range)	25.4 (6.75–280)	28.6 (4.17–57)	0.215
Recent contact with a person with a lasting cough	Present	8 (12)	2 (10)	1.000

Abbreviations: DTaP, acellular pertussis vaccine.
[a] Data expressed as n (%) unless otherwise indicated.
[b] The chi-square test, Fisher's exact test, or the Wilcoxon rank sum test was used as appropriate.

Table 2
Comparison of background characteristics between cases and controls.

Variable		Cases ($n=55$)	Controls ($n=90$)	P value[b]
		n (%)[a]	n (%)[a]	
Age (years)	Median (range)	9.6 (0.5–27.5)	9.7 (0.3–25.1)	0.912
Sex	Male	22 (40)	30 (33)	0.417
Number of DTaP vaccinations	0	7 (13)	3 (3)	0.061
	1–3	3 (5)	6 (7)	
	4	45 (82)	81 (90)	
Underlying illnesses	Present	21 (38)	21 (23)	0.056
History of steroid treatment	Present	10 (18)	5 (6)	0.015
Total room space in the house (m^2)	Median (range)	70.0 (24.75–200)	104.0 (25–839)	0.024
Number of family members	Median (range)	4.0 (1–7)	4.0 (1–7)	0.613
Room space per person (m^2)	Median (range)	21.7 (8.0–140)	25.9 (4.17–280)	0.039
Recent contact with a person with a lasting cough	Present	17 (31)	10 (11)	0.003

Abbreviations: DTaP, acellular pertussis vaccine.
[a] Data expressed as n (%) unless otherwise indicated.
[b] The chi-square test, Fisher's exact test, or the Wilcoxon rank sum test was used as appropriate.

Table 3
Adjusted ORs of DTaP vaccination and selected variables for pertussis: unconditional and conditional logistic regression models.

Variable		Unconditional model		Conditional model	
		n (%) or median; cases/controls	OR (95%CI) P value	n (%) or median; cases/controls	OR (95%CI) P value
DTaP vaccination	None	7 (13)/3 (3)	1.00 (ref.)	4 (12)/3 (4)	1.00 (ref.)
	Received	48 (87)/87 (97)	0.20 (0.04–0.97) 0.045	29 (88)/65 (96)	0.15 (0.01–1.80) 0.133
Number of vaccinations	1–3	3 (5)/6 (7)	0.15 (0.02–1.24) 0.078	1 (3)/5 (7)	0.12 (0.01–1.91) 0.133
	4	45 (82)/81 (90)	0.22 (0.04–1.05) 0.057 (Trend $P=0.098$)	28 (85)/60 (88)	0.20 (0.01–4.73) 0.319 (Trend $P=0.249$)
History of steroid treatment	Present	10 (18)/5 (6)	3.98 (1.17–13.6) 0.027	6 (18)/4 (6)	8.23 (1.25–54.3) 0.029
Total room space in the house (m^2)	1 m^2 Increased	70.0/104.0	0.99 (0.98–0.99) 0.036	74.0/108.5	0.98 (0.96–0.99) 0.019
Number of family members	1 Person increased	4.0/4.0	1.12 (0.81–1.55) 0.486	4.0/4.0	1.85 (0.93–3.68) 0.080
Recent contact with a person with a lasting cough	Present	17 (31)/10 (11)	4.62 (1.73–12.4) 0.002	10 (30)/7 (10)	4.44 (1.10–18.0) 0.037

Abbreviations: CI, confidence interval; DTaP, acellular pertussis vaccine; OR, odds ratio.

Table 4
Adjusted odds ratios of DTaP vaccination for pertussis: several sensitivity analyses using unconditional logistic regression models[a].

Analyzed subjects	≥1 Dose of DTaP vaccination (ref. none)	4 Doses of DTaP vaccination (ref. none)
	OR (95%CI) P value	OR (95%CI) P value
All subjects (55 cases/90 controls)	0.20 (0.04–0.97) 0.045	0.22 (0.04–1.05) 0.057
Limited to subjects whose vaccination status could be confirmed by immunization records (52 cases/88 controls)	0.21 (0.04–0.99) 0.049	0.22 (0.05–1.08) 0.063
Limited to subjects aged less than 18 (43 cases/73 controls)	0.22 (0.04–1.18) 0.077	0.22 (0.04–1.25) 0.088
Laboratory confirmed cases vs. all controls (39 cases/90 controls)	0.25 (0.05–1.39) 0.114	0.28 (0.05–1.62) 0.156
All cases vs. friend controls (55 cases/69 controls)	0.30 (0.06–1.42) 0.130	0.29 (0.06–1.38) 0.120

Abbreviations: CI, confidence interval; DTaP, acellular pertussis vaccine; OR, odds ratio.
[a] Adjusted for history of steroid treatment, room space in the house, number of family members, recent contact with a person with a lasting cough, and matching variables (age and sex).

Table 5
Adjusted xORs of 4-dose vaccines for pertussis, according to time since the fourth dose.

Variable	Cases (n = 51)[a]	Controls (n = 84)	Unconditional model[b]
	n (%)	n (%)	OR (95%CI) P value
Number of vaccinations, Time since the fourth dose for 4-dose vaccinees			
0 Doses	7 (14)	3 (4)	1.00 (Ref.)
4 Doses, <7.7 years	22 (43)	41 (49)	0.22 (0.04–1.07) 0.060
4 Doses, 7.7–24.2 years	22 (43)	40 (48)	0.18 (0.03–1.13) 0.067 (Trend P = 0.124)
0 Doses	7 (14)	3 (4)	1.00 (Ref.)
4 Doses, <5.8 years	17 (33)	28 (33)	0.24 (0.05–1.23) 0.087
4 Doses, 5.8–9.1 years	12 (24)	27 (32)	0.14 (0.02–0.87) 0.035
4 Doses, 9.2–24.2 years	15 (29)	26 (31)	0.11 (0.01–1.02) 0.052 (Trend P = 0.057)

Abbreviations: CI, confidence interval; DTaP, acellular pertussis vaccine; OR, odds ratio.
[a] Since one case did not provide the time of the fourth dose vaccination, the case was not included in the analysis.
[b] Adjusted for history of steroid treatment, room space in the house, number of family members, recent contact with a person with a lasting cough, and matching variables (age and sex).

effectiveness for decreasing the secondary attack rates in children aged 0 to 6 years [15]. Another population-based case control study, which was conducted during a non-epidemic period (1999–2001), showed that the effectiveness of 3 or 4 vaccinations for physician-diagnosed pertussis was 96% (95%CI: 54–99%) among children aged less than 6 years [16]. In the other retrospective cohort study among university students, which was conducted just after the pertussis outbreak ended (2010), the reported vaccine effectiveness was 52% for probable pertussis [14]. Taken together, the observed effectiveness might be higher in a study during a non-epidemic period than during an outbreak.

In addition, effectiveness might vary according to the age distribution in the study subjects. Previous studies reported the possibility that DTaP vaccine effectiveness was waning by time since the final dose [9–13]. In the present study, however, there was no waning in effectiveness by time since the final dose, and effectiveness was observed even 9 years or more after the final dose, as shown in Table 4. Although the reason for the discrepancy across studies was not clear, the following explanations could be considered: (1) lower statistical power to detect waning effectiveness in the present study; and (2) the present study's results might be affected by the previous booster effects from undiagnosed natural infection in the community. Particularly with respect to the latter explanation, Okada et al. reported that 58% of the unvaccinated population had PT-IgG antibody of 10 EU/mL or more, and 79% had pertussis filamentous hemagglutinin antibody of 10 EU/mL or more, which suggested the presence of undiagnosed natural infection [17]. Furthermore, a pertussis epidemic had sporadically occurred during the late 2000s in Japan. Thus, the present results may have been affected by the previous booster effects from undiagnosed natural infection. In fact, subjects with incomplete vaccination (1–3 doses) also had 85% effectiveness for preventing pertussis in the present study. When we consider that the effectiveness of incomplete vaccination might be explained by the previous booster effects from undiagnosed natural infection, this seems reasonable.

As for the other associated factors, the present study suggested that subjects with a history of steroid treatment had a higher risk for pertussis (Table 3). To the best of our knowledge, no study has reported the association between history of steroid treatment and pertussis. However, some studies showed a higher risk for pertussis in patients with asthma [18,19], who often receive steroid treatment. In addition, several studies reported that steroid treatment was a risk factor for respiratory infections such as pneumonia [20] and influenza [21]. Taken together, it is therefore plausible that a history of steroid treatment also increased the individual risk for infection with other respiratory pathogens such as pertussis.

In the present study, living in a smaller room space and having recent contact with a person with a lasting cough were also related to pertussis, independent of vaccination status or history of steroid treatment. Although the present study included friend controls, the proportion of "having recent contact with a person with a lasting cough" was only 12% in friend controls, suggesting that contact with present study cases (i.e., physician-diagnosed pertussis) was not common among friend controls. This might be partly explained by the fact that most of the present study cases were absent from school after symptom onset. In the light of previous studies, pertussis outbreaks often occurred in crowded situations such as in schools [14,22], families [15], or soldiers [23]. Furthermore, some studies reported that subjects who had contact with a person with a pertussis-like cough had a higher risk for pertussis infection [23–25]. Thus, the present results agreed with the previous findings. These results suggest that increased susceptibility to pertussis in a crowded situation or increased opportunities on contact with possible pertussis patients would be related to pertussis infection.

The present case-control study had a unique design that included friend controls. However, some might think that hospital controls would have been preferable, because cases were selected from hospital patients. To examine vaccine effectiveness, however, it is very important to consider the likelihood of exposure to the pathogen, which is a necessary cause for infectious disease. Particularly in the case of pertussis, disease occurrence is sporadic, which is different from common infectious diseases such as influenza. For pertussis, traditional hospital controls or general population controls might not have had contact with the pathogen. Vaccine effectiveness should be estimated under the assumption that controls have a similar potential for exposure to the pathogen as cases. It is therefore considered that friend controls would be among the most suitable controls in terms of sharing a similar potential for exposure to the pathogen as cases. However, friend controls might have similar background characteristics to those of cases, such as socioeconomic status, religious beliefs, and even probably vaccination status, which might contribute to the underestimation of VE. We considered that underestimation of VE, if any, would not affect the plausibility of the study results, and therefore decided to use friend controls.

When interpreting the present results, however, the following limitations should be considered. First, insufficient statistical power due to the small sample size is obviously important. This limitation made it difficult to detect significant vaccine effectiveness. However, pertussis cases in Japan have decreased substantially not only at the collaborating hospitals in the present study, but also in all parts of Japan during the study period [5]. Thus, it was thought

that obtaining more subjects would be impossible. Second, there may be concern that changing the protocol with respect to control selection during the study period might have had some effect on the present results. Of particular concern was that hospital controls might not have had contact with the pathogen, but the proportion of "recent contact with a person with a lasting cough" was found to be similar between friend controls and hospital controls. On the other hand, hospital controls were younger and had more underlying illnesses with steroid treatment than friend controls, which might have affected to the results. To consider the effect of including hospital controls during the study period, however, when analyses were limited to friend controls and cases, the ORs of DTaP vaccination were almost unchanged, and 95% CIs became wider, suggesting that including hospital controls increased statistical power (Table 4). Third, the present results were obtained after adjustment for potential confounders (i.e., history of steroid treatment, total room space in the house, number of family members, recent contact with a person with a lasting cough), but the effects of other confounding factors, such as socioeconomic status, birth order, and school attendance, could not be considered.

In conclusion, the present results support that DTaP vaccination in the routine immunization program in Japan had a preventive effect against infection with pertussis. Effectiveness was observed even 9 or more years after the fourth dose. However, observed effectiveness in the present study might have included not only genuine vaccine effectiveness, but also the effects of previous booster effects from undiagnosed natural infection in the community. To consider whether adding a booster dose of vaccination for adolescents is needed, results from descriptive epidemiological studies of pertussis outbreaks, seroepidemiologic studies, and further large-scale studies about vaccine effectiveness, if possible, are needed.

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References

[1] Kimura M, Kuno-Sakai H. Developments in pertussis immunization in Japan. Lancet 1990;336:30–2.
[2] Kimura M, Kuno-Sakai H. Current epidemiology of pertussis in Japan. Pediatr Intfect Dis J 1990;9:705–9.
[3] Kuno-Sakai H, Kimura M. Safety and efficacy of acellular pertussis vaccine in Japan, evaluated by 23 years of its use for routine immunization. Pediatr Int 2004;46:650–5.
[4] Noble GR, Bernier RH, Esber EC, Hardegree MC, Hinman AR, Klein D, et al. Acellular and whole-cell pertussis vaccines in Japan. JAMA 1987;257:1351–6.
[5] National Institute of Infectious Diseases. Fact sheet for pertussis vaccine [in Japanese]. Available at ⟨http://www.mhlw.go.jp/stf/shingi/2r9852000000bx23-att/2r9852000000byfg.pdf⟩ (accessed 11 July 2014).
[6] Wendelboe AM, van Rie A, Salmaso S, Englund JA. Duration of immunity against pertussis after natural infection or vaccination. Pediatr Intfect Dis J 2005;24:S58–61.
[7] Tartof SY, Lewis M, Kenyon C, White K, Osborn A, Liko J, et al. Waning immunity to pertussis following 5 doses of DTaP. Pediatrics 2013;131:e1047.
[8] Centers for Disease Control and Prevention, Advisory Committee on Immunization Practices (ACIP). Recommended immunization schedule for persons aged 0 through 18 years—United States, 62. United States: MMWR; 2013. p. 2–8.
[9] Tindberg Y, Blennow M, Granstrom M. A ten year follow-up after immunization with a two component acellular pertussis vaccine. Pediatr Infect Dis J 1999;18:361–5.
[10] Klein NP, Bartlett J, Rowhani-Rahbar A, Fireman B, Baxter R. Waning protection after fifth dose of acellular pertussis vaccine in children. N Engl J Med 2012;367:1012–9.
[11] Misegades LK, Winter K, Harriman K, Talarico J, Messonnier NE, Clark TA, et al. Association of childhood pertussis with receipt of 5 doses of pertussis vaccine by time since laset vaccine dose, California, 2010. JAMA 2012;308:2126–32.
[12] Witt MA, Katz PH, Witt DJ. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. Clin Infect Dis 2012;54:1730–5.
[13] Sin MA, Zenke R, Onckendorf R, Littmann M, Jorgensen P, Hellenbrand W. Pertussis outbreak in primary and secondary schools in Ludwigslust, Germany demonstrating the role of waning immunity. Pediatr Infect Dis J 2009;28:242–4.
[14] Hara M, Okada K, Nakano T, Ohfuji S, Sunagawa T, Kamachi K, et al. Pertussis outbreak in university students and evaluation of acellular pertussis vaccine effectiveness in Japan. In: Hirota Y, editors. Annual Report of Research Committee on Analytic Epidemiology for Vaccines (in Japanese). Tokyo: Research on Emerging and Re-emerging Infectious Diseases, The Ministry of Health and Labor and Welfare of Japan, 2011, p. 174-182.
[15] Aoyama T, Murase Y, Kato T, Iwata T. Efficacy of an acellular pertussis vaccine in Japan. J Pediatr 1985;107:180–3.
[16] Okada K, Ohashi Y, Matsuo F, Uno S, Soh M, Nishima S. Effectiveness of an acellular pertussis vaccine in Japanese children during a non-epidemic period: a matched case-control study. Epidemiol Infect 2009;137:124–30.
[17] Okada K, Ueda K, Morokuma K, Kino Y, Tokunaga K, Nishima S. Seroepidemiologic study on pertussis, diphtheria, and tetanus in the Fukuoka area of southern Japan: seroprevalence among persons 0–80 years old and vaccination program. Jpn J Infect Dis 2004;57:67–71.
[18] Liu BC, Mclntyre P, Kaldor JM, Quinn HE, Ridda I, Banks E. Pertussis in older adults: prospective study of risk factors and morbidity. Clin Infect Dis 2012;55:1450–6.
[19] Capili CR, Hettinger A, Rigelman-Hedberg N, Fink L, Boyce T, Lahr B, et al. Increased risk of pertussis in patients with asthma. J Allergy Clin Immunol 2012;129:957–63.
[20] Yawn BP, Li Y, Tian H, Zhang J, Arcona S, Kahler KH. Inhaled corticosteroid use in patients with chronic obstructive pulmonary disease and the risk of pneumonia: a retrospective claims data analysis. Int J COPD 2013;8:295–304.
[21] Recommendations of the Advisory Committee on Immunization Practices (ACIP). Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the ACIP-United States, 2013–14. MMWR; 62 (RR07):1–43.
[22] Berger F, Njamkepo E, Minaberry S, Mayet A, Haus-Cheymol R, Verret C, et al. Investigation on a pertussis outbreak in a military school: risk factors and approach to vaccine efficacy. Vaccine 2010;28:5147–52.
[23] Waters V, Jamieson F, Richardson SE, Fikelstein M, Wormsbecker A, Halperin SA. Outbreak of atypical pertussis detected by polymerase chain reaction in immunized preschool-aged children. Pediatr Infect Dis J 2009;28:582–7.
[24] Klement E, Uliel L, Engel I, Hasin T, Yavzori M, Orr N, et al. An outbreak of pertussis among young Israeli soldiers. Epidemiol Infect 2003;131:1049–54.
[25] Izurieta HS, Kenyon TA, Strebel PM, Baughman AL, Shulman ST, Wharton M. Risk factors for pertussis in young infants during an outbreak in Chicago in 1993. Clin Infect Dis 1996;22:503–7.

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Reactogenicity of trivalent inactivated influenza vaccine in young children: Pronounced reactions by previous successive vaccinations

Chika Okada[a,*,1], Megumi Fujieda[a,1], Wakaba Fukushima[a], Satoko Ohfuji[a], Kyoko Kondo[a], Akiko Maeda[a], Takashi Nakano[b], Masaro Kaji[c], Yoshio Hirota[a], for the Influenza Vaccine Epidemiology Study Group[2]

[a] Department of Public Health, Osaka City University Faculty of Medicine, 1-4-3 Asahi-machi, Abeno-ku, , Osaka, 545-8585, Japan
[b] National Mie Hospital, 357 Ozato-Kubota, Tsu, Mie, 514-0125, Japan
[c] Kurume University, 67 Asahi-machi, Kurume-shi, Fukuoka, 830-0011, Japan

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ABSTRACT

In order to assess factors associated with reactogenicity of trivalent inactivated influenza vaccine (IIV3) among young children, data on 1538 vaccinees aged 0–5 years in a previous vaccine effectiveness study were analyzed.

The most frequent reaction was redness (19%), followed by induration, swelling, itching, and pain (6–12%); there were no serious adverse events. For some local reactions, multivariate analyses indicated associations of younger age, preschool attendance, presence of siblings, and allergy with lower risk, and use of thinner needles with higher risk. Most notably, administration of one or more IIV3 vaccines during the previous 3 seasons was positively associated with each local reaction (adjusted odds ratios: 3.6–5.4). For subjects aged ≥3 years, prior successive annual vaccinations were associated with substantially increased local reactions, with clear dose-response relationships (*P* for trend: <0.001 for each); for example, an 9.8-fold greater risk of swelling following three successive annual vaccinations before the study season.

1. Introduction

Administration of the influenza vaccine is the most effective measure to prevent progression to severe complications and mortality from the influenza virus [1]. However, vaccination has caused adverse events in a higher proportion compared to placebo [2,3]; that is, reactogenicity is inevitable. It is important when promoting vaccination to explain the risk of reactogenicity to provide the opportunity of vaccination with improved expectations.

Previous studies regarding factors associated with reactogenicity after influenza vaccination are inconsistent. For example, it was reported that females manifested significantly more local reactions than males [4], but another study showed that sex was not significantly associated with systemic and local reactions [5]. Such evidence regarding young children is very limited, although some studies of other vaccines, such as diphtheria–tetanus–acellular pertussis vaccine or human papillomavirus vaccine, were reported [6,7]. Accordingly, it is necessary to accumulate more data regarding reactogenicity after influenza vaccination in young children.

In Japan, young children were reportedly the most frequently affected by both serious and non-serious local or systemic reactions after receiving influenza vaccine [8,9], although vaccination for this age group is recommended by the United States Center for Disease Control and Prevention since they have a relatively high rate of influenza-associated hospitalization [1]. Therefore, we assessed the reactogenicity of the influenza vaccine and associated factors in children, using the data that we had previously collected to evaluate its effectiveness [10].

2. Materials and methods

2.1. Study subjects and vaccination

The study subjects were 1569 Japanese children aged less than 6 years who received trivalent inactivated influenza vaccine (IIV3) on parental request during the 2002/03 season at one of 54 pediatric

* Corresponding author. Tel.: +81 6 6645 3756; fax: +81 6 6645 3757.
E-mail address: chikaokda@gmail.com (C. Okada).
[1] Both authors contributed equally to this work.
[2] Other members of the Study Groups are listed in the Appendix.

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clinics in Japan. They were vaccinees in our previous cohort study to assess influenza vaccine effectiveness [10].

Vaccinations were performed by the pediatrician in charge at each clinic using commercial, non-adjuvanted, inactivated influenza vaccines that included the following strains: A/New Caledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2), and B/Shandong/7/97. These vaccines contained 30 μg/mL of hemagglutinin (HA) from each strain. Subjects received two doses by subcutaneous injections into their arms of 0.1 mL for children aged less than 1 year, or 0.2 mL for those aged 1–5.9 years, in accordance with the guideline for vaccination in Japan at that time. All parents or guardians gave informed consent. The study protocol was approved by the ethics committee of the Osaka City University Faculty of Medicine.

2.2. Information collection

Data on baseline characteristics were obtained from responses to 2 structured questionnaires. One was answered by the parents or guardians and included questions regarding sex, age, history of IIV3 vaccination during the previous 3 seasons, preschool attendance, and number of family members and siblings. The other was completed by the physician and provided information concerning body weight, underlying diseases (heart disease, kidney disease, diabetes, anemia, bronchial asthma, tonsillitis, hives, atopy, and allergy), needle gauge size used, and the vaccine manufacturer.

The parents or guardians were asked to report prospectively, by indicating "no" or "yes" on a postal questionnaire, the occurrence of local and systemic adverse events within 48 h after vaccination. Local reactions included redness, swelling, induration, itching, and pain. Systemic reactions were fever (defined at 0.5 °C intervals) and rash. Information on medical office visits due to the adverse events was also solicited.

2.3. Statistical analysis

After excluding 31 children (4 for age ≥6 years; 22 for vaccine doses in violation of protocol; and 5 for no information on adverse events), data from 1538 vaccinees were analyzed. Although the parents or guardians of 171 children (11%) failed to answer one or more questionnaire items on adverse events, they were included in the analyses of each adverse event in order to utilize the maximum of available information.

The frequencies of adverse events were compared after dose 1 and dose 2 using McNemar's test. The odds ratios (ORs) for associations of baseline characteristics with adverse events and their 95% confidence intervals (CIs) were calculated using the logistic regression model. To select the explanatory variables for the multivariate model, we used a stepwise method involving variables that had a statistically significant association, by univariate analysis, with one of the adverse events. Seven selected variables at this step were age, preschool attendance, history of IIV3 vaccination during the previous 3 seasons, presence of siblings, allergy, needle gauge size used, and the vaccine manufacturer. The final model also included sex in addition to these 7 variables.

For comparison, subjects aged less than 2 years were combined into a single category because only a few subjects less than 1 year of age reported adverse events. The needle gauge size was divided by approximate tertiles (23-25G/26G/27-30G). In calculating ORs for age and manufacturer, referent categories were assigned to the levels in which the maximum numbers of subjects were distributed.

For univariate and multivariate analyses, we used the adverse events after dose 1 as outcome measures since they were generally more frequent as compared to those occurring after dose 2 (e.g., $P = 0.02$ for redness and rash). A P value <0.05 was considered statistically significant. All statistical analysis was performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Table 1
Characteristics of the study population ($N = 1538$).

	n (%) or median (range)	
Boy	793	(52)
Age (years)		
<1.0	25	(2)
1.0–1.9	229	(15)
2.0–2.9	352	(23)
3.0–3.9	369	(24)
4.0–4.9	316	(21)
5.0–5.9	247	(16)
Current body weight (kg)[a]	14.4	(6.9–30.0)
Preschool attendance (yes)	932	(61)
Sibling (yes)[b]	1096	(71)
Number of siblings[b]	1	(0–4)
Number of family members[b]	4	(2–45)
Influenza vaccination during the previous 3 seasons (yes)	1080	(70)
Underlying disease (yes)		
Heart disease	15	(1)
Kidney disease	5	(0)
Diabetes	0	(0)
Anemia	9	(1)
Bronchial asthma	191	(12)
Tonsillitis	34	(2)
Hives	57	(4)
Atopy	102	(7)
Allergy	106	(7)

[a] Missing information for 2 subjects.
[b] Residing in the same household.

3. Results

Table 1 lists the distribution of characteristics. The 3.0–3.9 years age group had the largest number of subjects (24%), and 70% of the children had a history of IIV3 vaccination during the previous 3 seasons. The frequent underlying diseases were bronchial asthma (12%), allergy (7%), and atopy (7%).

The occurrence of adverse events is presented in Table 2. About 25% of subjects reported one or more local reactions (hereinafter referred to as 'any local reaction') after dose 1 and dose 2. The most frequent local reaction was redness, followed by induration, swelling, itching, and pain. Systemic reactions (fever ≥37.5 °C and rash) were seen in 3% or fewer of subjects. Only one subject manifested high fever of ≥39.5 °C, which occurred after each dose. There were very few medical office visits related to reactions (for 3% of subjects with any local reaction after dose 1 and 1% after dose 2).

In univariate analyses (Table 3), significantly lowered ORs for local reactions were seen for the following variables: younger age (for each of the local reactions), preschool attendance (for redness), presence of siblings (for redness), allergy (for swelling), and C and

Table 2
Adverse events within 48 h after vaccination.

	After dose 1		After dose 2	
	n/N	(%)	n/N	(%)
Local reactions				
Any local reactions	394/1533	(26)	366/1503	(24)
Redness	285/1532	(19)	249/1503	(17)
Swelling	173/1531	(11)	157/1501	(11)
Induration	182/1531	(12)	173/1501	(12)
Itching	126/1531	(8)	122/1501	(8)
Pain	97/1532	(6)	90/1502	(6)
Systemic reactions				
Fever (≥37.5 °C)	42/1525	(3)	48/1481	(3)
Rash	25/1468	(2)	11/1433	(1)

Table 3
Odds ratios (95% confidence intervals) of selected variables for adverse events after dose 1 (univariate analyses).

Variable, category	Local reactions						Systemic reactions	
	Any	Redness	Swelling	Induration	Itching	Pain	Fever (≥37.5 °C)	Rash
No. of subjects†	1533	1532	1531	1531	1531	1532	1525	1468
Sex								
Girl (vs. boy)	1.1 (0.9–1.4)	1.1 (0.8–1.4)	0.9 (0.6–1.2)	1.1 (0.8–1.5)	1.2 (0.9–1.8)	1.3 (0.9–2.0)	0.7 (0.4–1.3)	0.7 (0.3–1.6)
Age (years)								
<2.0	0.3 (0.2–0.5)*	0.3 (0.2–0.5)*	0.3 (0.2–0.6)*	0.4 (0.2–0.7)*	0.1 (0.0–0.3)*	0.1 (0.0–0.4)*	0.4 (0.2–1.1)	1.2 (0.4–3.2)
2.0–2.9	0.9 (0.6–1.2)	1.0 (0.7–1.4)	1.0 (0.6–1.5)	0.9 (0.6–1.4)	0.6 (0.4–0.9)*	0.6 (0.3–1.2)	0.7 (0.3–1.5)	0.7 (0.3–2.0)
3.0–3.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
4.0–4.9	0.9 (0.6–1.2)	0.7 (0.5–1.1)	0.9 (0.6–1.4)	0.7 (0.4–1.1)	0.7 (0.4–1.1)	0.9 (0.5–1.6)	0.1 (0.0–0.6)*	0.1 (0.0–1.0)
5.0–5.9	1.1 (0.8–1.5)	0.7 (0.5–1.1)	1.0 (0.6–1.6)	1.0 (0.6–1.5)	0.9 (0.5–1.5)	1.4 (0.8–2.4)	0.6 (0.3–1.5)	0.3 (0.1–1.5)
Preschool attendance								
Yes (vs. no)	1.0 (0.8–1.3)	0.8 (0.6–0.9)*	1.0 (0.7–1.3)	1.1 (0.8–1.4)	1.2 (0.8–1.7)	1.7 (1.1–2.6)	1.2 (0.6–2.2)	0.5 (0.2–1.1)
Presence of siblings								
Yes (vs. none)	0.8 (0.6–1.0)	0.7 (0.5–0.9)*	0.8 (0.6–1.1)	0.9 (0.6–1.2)	1.2 (0.8–1.8)	1.2 (0.8–2.0)	0.8 (0.4–1.6)	0.6 (0.3–1.3)
Allergy								
Yes (vs. none)	1.0 (0.6–1.6)	0.8 (0.4–1.3)	0.3 (0.1–0.8)*	0.6 (0.3–1.2)	0.9 (0.4–1.9)	1.4 (0.7–2.9)	1.4 (0.5–4.1)	1.9 (0.6–6.5)
Vaccination during the previous 3 seasons								
Yes (vs.none)	5.4 (3.8–7.8)*	5.7 (3.7–8.9)*	6.0 (3.4–10)*	4.4 (2.7–7.2)*	6.9 (3.3–14)*	5.9 (2.7–13)*	1.4 (0.7–2.8)	0.8 (0.3–1.7)
Needle gauge size								
23–25G	1.0	1.0	1.0	1.00	1.0	1.0	1.0	1.0
26G	1.7 (1.3–2.3)*	2.2 (1.5–3.1)*	2.2 (1.4–3.5)*	1.9 (1.3–2.9)*	1.8 (1.1–2.9)*	1.0 (0.6–1.8)	0.8 (0.4–1.7)	2.8 (0.9–8.7)
27–30G	1.7 (1.2–2.2)*	2.0 (1.4–2.9)*	2.0 (1.3–3.1)*	1.7 (1.1–2.6)*	1.9 (1.2–3.2)*	1.1 (0.7–1.9)	0.9 (0.4–1.8)	1.6 (0.5–5.3)
Vaccine manufacturer								
A	0.9 (0.6–1.4)	1.0 (0.7–1.6)	1.1 (0.6–1.8)	0.9 (0.6–1.5)	0.7 (0.4–1.4)	1.4 (0.7–2.6)	0.4 (0.1–1.7)	1.6 (0.3–8.0)
B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C	0.5 (0.3–0.7)*	0.5 (0.3–0.8)*	0.6 (0.3–1.0)	0.4 (0.2–0.7)*	0.7 (0.4–1.1)	0.6 (0.3–1.2)	0.5 (0.2–1.4)	2.5 (0.7–8.2)
D	0.6 (0.5–0.8)*	0.6 (0.4–0.8)*	0.8 (0.5–1.2)	0.7 (0.5–1.0)	0.6 (0.4–0.9)*	1.0 (0.6–1.6)	0.9 (0.4–1.7)	3.2 (1.2–8.5)*

* Statistically significant.
† Effective for analyses.

D manufacturer (for any local reaction, redness, induration, and itching). On the other hand, elevated ORs with significance were shown for the following: IIV3 vaccination during the previous 3 seasons (for each of the local reactions) and use of the thinner 26G and 27–30G needles (for each of the local reactions except for pain). Regarding systemic reactions, significant ORs were indicated in the age 4.0–4.9 years group (for fever) and for the D manufacturer (for rash).

In multivariate analyses (Table 4), statistically significant ORs were observed for almost the same variables, but not for the same categories, as in univariate analyses. Notably, multivariate ORs of previous IIV3 vaccination for each local reaction still were the highest as compared to those of other variables (ORs = 3.6–5.4 for each of the local reactions). The use of thinner needles also had increased ORs (1.6–2.2) for any local reaction, redness, swelling, induration, and itching.

Based on the strong positive association between previous IIV3 vaccination and occurrence of local reactions, we further assessed the effect of successive annual vaccinations immediately before the present season (Table 5). Among those subjects aged ≥3 years with information on annual vaccination history during the previous 3 seasons (882 subjects), ORs for all local reactions elevated with increasing numbers of successive annual vaccinations: taking the example of "swelling", ORs of the preceding one, two, and three annual vaccinations, as compared to no vaccination history, were 4.8, 5.6, and 9.8, respectively, with clear dose-response relationships (*P* for trend: <0.001). Similar findings were also observed among those aged ≥2 years (1226 subjects).

Because our subjects for analyses were those who received the first dose, the selection bias may have been introduced if children who experienced an unpleasant event at the first dose were less likely to receive a second dose. In order to explore the possible influence of the bias, we repeated the multivariate analyses shown in Table 4 and Table 5 after excluding subjects who did not receive a second dose. The results were not meaningfully changed (data not shown).

4. Discussion

In this study population, aged less than 6 years, the most frequent local reaction was redness. Systemic reactions (fever and rash) were few. These findings are consistent with those of an earlier study in children aged 6–23 months, in which redness was frequent and fever was not common [11]. On the other hand, in some studies of influenza vaccine in children aged 6–35 months or 6–9 and 10–13 years, pain was the most common symptom [12,13]. These study subjects received the vaccine by intramuscular injection, which was different from our study (subcutaneous injection). Other subjects' characteristics including age and race might also explain the different findings. In the present study, it was also observed that most adverse events were not so severe as to require a medical office visit, and no serious events occurred. Thus, we consider that adverse events occurring in this study were well tolerated.

The relationship between sex and occurrence of any adverse event after influenza vaccination was not significant in this study. This result is consistent with those of a study of elderly people, which showed no significant relationship between sex and systemic or local reactions [5], although a meta-analysis of 14 studies found that female adults report more local reactions than do males [4]. To our knowledge, there has been no previous study that assessed the effect of sex on occurrence of adverse events to influenza vaccination in young children. With respect to other vaccines, a recent review article reports inconsistent results among children aged 4–6 years [6], as well as that adult females tended to report local reactions more frequently than do adult males [6,7]. One possible explanation is that sex hormone levels at the extremes of life may contribute to the different findings between adults and young children or elderly people.

A few previously reported studies of young children included analysis of the frequencies of adverse events with regard to various age levels, but the age categories used for comparison were too broad (e.g., 5–6 and 7–8 years, 6–9 and 10–13 years) [13,14]. When

Table 4
Odds ratios (95% confidence intervals) of selected factors for adverse events after dose 1 (multivariate analyses [a]).

Variable, category	Local reactions						Systemic reactions	
	Any	Redness	Swelling	Induration	Itching	Pain	Fever (≥37.5 °C)	Rash
No. of subjects[†]	1530	1529	1528	1528	1528	1529	1522	1465
Sex								
Girl (vs. boy)	1.1 (0.9–1.5)	1.1 (0.8–1.4)	0.9 (0.7–1.3)	1.1 (0.8–1.5)	1.3 (0.9–1.9)	1.4 (0.9–2.1)	0.7 (0.4–1.3)	0.7 (0.3–1.7)
Age (years)								
<2.0	0.6 (0.4–1.0)	0.6 (0.3–1.1)	0.7 (0.4–1.5)	0.8 (0.4–1.6)	0.2 (0.1–0.7)*	0.1 (0.0–0.7)*	0.6 (0.2–1.8)	0.9 (0.3–3.2)
2.0–2.9	0.8 (0.6–1.2)	0.9 (0.6–1.3)	0.9 (0.6–1.5)	1.0 (0.6–1.6)	0.6 (0.4–1.1)	0.7 (0.4–1.3)	0.7 (0.3–1.5)	0.6 (0.2–1.9)
3.0–3.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
4.0–4.9	0.9 (0.7–1.4)	0.8 (0.6–1.3)	1.0 (0.6–1.6)	0.7 (0.4–1.1)	0.7 (0.4–1.1)	0.9 (0.5–1.7)	0.1 (0.0–0.5)*	0.2 (0.0–1.2)
5.0–5.9	1.2 (0.8–1.8)	0.9 (0.6–1.4)	1.1 (0.7–1.9)	1.0 (0.6–1.6)	0.9 (0.5–1.6)	1.4 (0.8–2.5)	0.5 (0.2–1.3)	0.4 (0.1–1.9)
Preschool attendance								
Yes (vs. no)	0.7 (0.5–0.9)*	0.6 (0.5–0.9)*	0.8 (0.5–1.2)	1.0 (0.7–1.5)	0.9 (0.6–1.4)	1.0 (0.6–1.7)	1.6 (0.8–3.2)	0.8 (0.3–1.9)
Presence of siblings								
Yes (vs. none)	0.7 (0.5–0.9)*	0.7 (0.5–0.9)*	0.7 (0.5–1.1)	0.8 (0.6–1.2)	1.1 (0.7–1.7)	1.1 (0.7–1.8)	0.8 (0.4–1.5)	0.6 (0.3–1.4)
Allergy								
Yes (vs. None)	1.0 (0.6–1.5)	0.8 (0.4–1.4)	0.3 (0.1–0.8)*	0.6 (0.3–1.2)	0.9 (0.4–1.8)	1.3 (0.6–2.7)	1.5 (0.5–4.3)	1.8 (0.5–6.3)
Vaccination during the previous 3 seasons								
Yes (vs. none)	4.7 (3.2–7.0)*	5.3 (3.3–8.5)*	5.4 (2.9–10)*	4.1 (2.4–7.1)*	4.5 (2.1–9.5)*	3.6 (1.6–8.0)*	1.4 (0.6–3.1)	1.2 (0.4–3.3)
Needle gauge size								
23–25G	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
26G	1.6 (1.2–2.3)*	2.2 (1.5–3.2)*	2.1 (1.3–3.4)*	1.8 (1.2–2.9)*	1.6 (0.9–2.7)	1.0 (0.6–1.8)	0.7 (0.3–1.5)	3.4 (0.9–13)
27–30G	1.6 (1.2–2.3)*	2.0 (1.4–2.9)*	2.0 (1.3–3.3)*	1.8 (1.1–2.7)*	1.8 (1.1–3.0)*	1.2 (0.7–2.1)	0.8 (0.4–1.8)	2.4 (0.6–9.7)
Vaccine manufacturer								
A	1.1 (0.7–1.7)	1.3 (0.8–2.1)	1.3 (0.8–2.3)	1.1 (0.6–1.9)	0.9 (0.4–1.7)	1.5 (0.8–3.0)	0.3 (0.1–1.4)	2.0 (0.4–11)
B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C	0.5 (0.3–0.7)*	0.5 (0.3–0.8)*	0.6 (0.3–1.0)	0.4 (0.2–0.7)*	0.7 (0.4–1.2)	0.6 (0.3–1.3)	0.5 (0.2–1.4)	2.3 (0.7–7.7)
D	0.7 (0.6–1.0)	0.7 (0.5–1.0)	1.0 (0.6–1.4)	0.8 (0.5–1.2)	0.7 (0.4–1.2)	1.2 (0.7–2.0)	0.8 (0.4–1.7)	2.8 (1.0–8.0)

* Statistically significant.
[†] Effective for analyses.
[a] All variables in the table were included in the model.

categorized by 1-year intervals in the present study, a significant negative association with itching, pain, and fever was seen in some of the groups. Future studies assessing the association between age and adverse events among young children may need to incorporate analysis of smaller age intervals.

This study indicates that preschool attendance and presence of siblings significantly decreased the ORs for any local reaction and redness. To the best of our knowledge, there has been no previous study with a similar result. This might be most appropriately interpreted as a tendency for guardians of children with such characteristics to be less likely to report adverse events. It is important to note that the ORs for 'any local reaction' and 'redness' tend to overestimate the real associations, since the incidence proportions of these outcomes were as large as 20%.

In the present study, use of thinner needles was significantly more likely to be associated with any local reaction, redness, swelling, induration, and itching than use of the thickest needle. A previous study on the diphtheria, pertussis, and tetanus (DPT) vaccine reported that use of longer needles caused fewer local reactions in comparison to shorter needles [15–17]. This finding was interpreted by equating shorter needles with thinner needles, and therefore it was suggested that the greater ensuing pressure enhances the local reaction [18]. Subsequent comparisons of use of needles with the same length but different thicknesses found no significant differences in resulting local reactions [19]. However, whether the effect was due to the needle length or thickness could not be ascertained in this study, since there were no data regarding the length of the needles. In addition, other factors including site or route (intramuscular or subcutaneous) of injection reportedly affect the reactogenicity [20,21].

We found that having one or more IIV3 vaccinations during the previous 3 seasons increased the risk for all local reactions. One

Table 5
Odds ratios (95% confidence intervals) of successive annual vaccinations for local reactions (multivariate analyses [a]).

History of successive annual vaccinations during the preceding seasons	Any	Redness	Swelling	Induration	Itching	Pain
Subjects aged ≥3 years (*n*)[†]	879	878	878	879	879	879
No history	1.0	1.0	1.0	1.0	1.0	1.0
With history in the preceding						
1 season	3.1 (1.7–5.8)	3.3 (1.5–7.2)	4.8 (1.6–14)	2.5 (1.1–5.7)	1.8 (0.7–4.8)	2.3 (0.8–6.8)
2 seasons	5.3 (2.9–9.6)	5.2 (2.5–11)	5.6 (2.0–16)	2.8 (1.3–6.2)	3.8 (1.6–9.2)	4.3 (1.6–11)
3 seasons	6.5 (3.5–12)	7.1 (3.3–15)	9.8 (3.4–29)	4.3 (1.9–9.8)	4.6 (1.8–11)	4.4 (1.6–12)
(*P* for trend)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Subjects aged ≥2 years (*n*)[†]	1222	1221	1220	1221	1221	1222
No history	1.0	1.0	1.0	1.0	1.0	1.0
With history in the preceding						
1 season	3.5 (2.2–5.7)	3.9 (2.2–7.0)	4,1 (1.9–9.0)	3.6 (1.8–7.0)	3.1 (1.2–7.6)	2.3 (1.0–5.6)
2 seasons	6.4 (4.0–10)	7.4 (4.1–13)	6.9 (3.2–15)	4.6 (2.4–9.0)	7.1 (3.0–17)	4.2 (1.9–9.7)
(*P* for trend)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[†] Effective for analyses.
[a] Adjusted for sex, age (categorical variable with 1-year interval), preschool attendance, presence of siblings, allergy, needle gauge size (23–25G/26G/27–30G) and vaccine manufacturers (4 companies).

previous report suggested that subjects with a history of IIV3 vaccination were more likely to report redness, although this result was not significant [22]. On the other hand, in previous studies among young children or elderly people, use of multivariate analysis failed to detect significant association between previous vaccination and systemic or local reactions [5,23]. In further exploring the effect of increasing numbers of successive annual vaccinations before the present season, we demonstrated an association with increased local reactions, with clear dose–response relationships. An earlier study of healthy adults in 1977, in which a similar hypothesis was tested, showed that increased numbers of previous vaccinations did not elevate the occurrence of adverse events [24].

The possible mechanism by which successive annual vaccinations increase the occurrence of local reactions has not been discussed extensively. A study of DPT vaccination revealed that local reactions were more frequent after the booster dose than after the primary vaccination, and that the serum level of pertussis toxin-specific immunoglobulin E antibodies was higher after the booster dose [25], which might provide some insight into the present findings.

Major strengths of this study include its prospective design and large cohort. In addition, precise analysis of factors associated with adverse events was possible, because a variety of information on characteristics of subjects had been collected.

This study has the following limitations. First, because we did not collect information regarding the severity of the reactions, it is difficult to compare our results with the results of other studies in which a specific grading scale for adverse events was used. Secondly, the findings obtained from the present study, using data collected when Japanese guidelines on vaccine doses for children had not been revised, cannot be directly compared with more current results in Western countries and in present-day Japan. Vaccines with high HA content have been reported to cause both systemic and local reactions more frequently compared to the lower HA-content vaccines used in the current study [11,26].

In conclusion, we found that adverse events after IIV3 vaccination among young children were mainly local reactions and not serious events. Several characteristics of subjects, including younger age, preschool attendance, presence of siblings, and allergy were associated with lower risk of local reaction, and IIV3 vaccinations during the previous 3 seasons were associated with higher risk. Use of a thinner needle was also significantly associated with a higher risk for some of the local reactions. Of note is that further research is needed to confirm our finding of positive trends for substantially increased local reactions in those with a history of prior successive annual vaccinations.

Appendix.

Other members of the Influenza Vaccine Epidemiology Study Group are: Drs. Tatsuru Yamanaka and Shuji Nakata in Hokkaido Prefecture; Drs. Yuhei Takasago, Mitsuo Kamihara, Yukiko Usui, Shuka Watanabe, Toshiko Yamaguchi, Shinji Yoshida, Asaka Nishijima, Tsuneji Kanno, Hiroyasu Wada, Eiji Ogawa and Kazuhiko Suzuki in Iwate Prefecture; Drs. Takamitsu Matsudaira, Shunsuke Numaguchi, Noriyuki Wada, Kinjiro Kodaira, Takayoshi Yamada and Akira Kamikawa in Tokyo Prefecture; Drs. Hitoshi Ochiai, Ritsue Nii, Naoki Yasuda, Takashi Kato, Masakazu Umemoto and Masahiro Watanabe in Mie Prefecture; Drs. Urara Kohdera, Katsuhiko Kidera, Fumiyoshi Yamaue, Masanobu Mantani, Michiaki Hayashida, Rentaro Abumi, Yuko Fukuda, Michiko Hatano, Kazuo Wada, Chikara Nakahama, Yoshiyuki Tanaka and Kyoko Takizawa in Osaka Prefecture; Drs. Takao Nagai, Takamichi Mukaida, Tetsushi Inoue, Junji Suzue, Fumihiko Hamada, Akira Takehiro, Atsuko Nishioka, Hitoshi Jinnai, Takuji Fujisawa and Kenkichi Sasaki in Shikoku region; Drs. Yoshio Takasaki, Shizuo Shindo, Naoki Tsumura and Yuji Yamashita in Fukuoka prefecture; and Drs. Yoshio Ohgimi and Yoshinobu Goya in Okinawa prefecture.

References

[1] Centers for Disease Control and Prevention. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), United States. MMWR Recomm Rep 2014:1–43, 2013;62 (RR-7).
[2] Jefferson T, Rivetti A, Di Pietrantonj C, Demicheli V, Ferroni E. Vaccines for preventing influenza in healthy children. Cochrane Database Syst Rev 2012;8:CD004879.
[3] Liang X-F, Wang H-Q, Wang J-Z, Fang H-H, Wu J, Zhu F-C, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2010;375:56–66.
[4] Beyer WE, Palache AM, Kerstens R, Masurel N. Gender differences in local and systemic reactions to inactivated influenza vaccine, established by a meta-analysis of fourteen independent studies. Eur J Clin Microbiol Infect Dis 1996;15(1):65–70.
[5] Govaert TM, Dinant GJ, Aretz K, Masurel N, Sprenger MJ, Knottnerus JA. Adverse reactions to influenza vaccine in elderly people: randomised double blind placebo controlled trial. BMJ 1993;307:988–90.
[6] Cook IF. Sex differences in injection site reactions with human vaccines. Hum Vaccin 2009;5(7):441–9.
[7] Petousis-Harris H, Poole T, Stewart J, Turner N, Goodyear-Smith F, Coster G, et al. An investigation of three injections techniques in reducing local injection pain with a human papillomavirus vaccine: a randomized trial. Vaccine 2013;31:1157–62.
[8] Pharmaceutical and Food Safety Bureau Ministry of Health, Labour and Welfare. Summary of Report on Adverse Reactions to the Influenza A (H1N1) Vaccine in the 2010 Season. Pharmaceuticals and Medical Devices Safety Information, 284; 2011. p. 12–7.
[9] Pharmaceutical and Food Safety Bureau Ministry of Health, Labour and Welfare. Summary of Report on Adverse Reactions to the Influenza Vaccine in the 2011 Season. Pharmaceuticals and Medical Devices Safety Information, 294; 2012. p. 8–12.
[10] Fujieda M, Maeda A, Kondo K, Kaji M, Hirota Y. Inactivated influenza vaccine effectiveness in children under 6 years of age during the 2002–2003 season. Vaccine 2006;24:957–63.
[11] Skowronski DM, Hottes TS, Chong M, De Serres G, Scheifele DW, Ward BJ, et al. Randomized controlled trial of dose response to influenza vaccine in children aged 6–23 months. Pediatrics 2011;128(2):e276–89.
[12] Scheifele DW, Ward BJ, Dionne M, Vanderkooi O, Langley JM, Dobson S, et al. Evaluation of adjuvanted pandemic H1N1(2009) influenza vaccine after one and two doses in young children. Pediatr Infect Dis J 2011;30: 402–7.
[13] Schmidt-Ott R, Schwarz T, Haase R, Sander H, Walther U, Fourneau M, et al. Immunogenicity and reactogenicity of a trivalent influenza split vaccine in previously unvaccinated children aged 6–9 and 10–13 years. Vaccine 2007;26:32–40.
[14] Neuzil KM, Jackson LA, Nelson J, Klimov A, Cox N, Bridges CB, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5–8-year-old children. J Infect Dis 2006;194: 1032–9.
[15] Diggle L, Deeks J. Effect of needle length on incidence of local reactions to routine immunisation in infants aged 4 months: randomised controlled trial. BMJ 2000;321:931–3.
[16] Diggle L, Deeks JJ, Pollard AJ. Effect of needle size on immunogenicity and reactogenicity of vaccines in infants: randomised controlled trial. BMJ 2006;333:571–4.
[17] Jackson LA, Starkovich P, Dunstan M, Yu O, Nelson J, Dunn J, et al. Prospective assessment of the effect of needle length and injection site on the risk of local reactions to the fifth diphtheria-tetanus-acellular pertussis vaccination. Pediatrics 2008;121(3):e646–52.
[18] Watson M. Needle length and incidence of local reactions to immunization. Needle gauge is more important than needle length. BMJ 2001;322:492.
[19] Bharti B, Grewal A, Kalia R, Pathak P. Vaccine related reactogenicity for primary immunization: a randomized controlled trial of 23 (wider) vs. 25 (narrower) gauge needles with same lengths. Indian J Pediatr 2010;77:1241–6.
[20] Petousis-Harris H. Vaccine injection technique and reactogenicity-evidence for practice. Vaccine 2008;26:6299–304.
[21] Herzog C. Influence of parenteral administration routes and additional factors on vaccine safety and immunogenicity: a review of recent literature. Expert Rev Vaccines 2014;13(3):399–415.
[22] Englund JA, Walter EB, Gbadebo A, Monto AS, Zhu Y, Neuzil KM. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. Pediatrics 2006;118(3):e579–85.

[23] Van Buynder PG, Frosst G, Van Buynder JL, Tremblay FW, Ross A, Jardine C, et al. Increased reactions to pediatric influenza vaccination following concomitant pneumococcal vaccination. Influenza Other Respir Viruses 2013;7(2): 184–90.
[24] Hennessen W, Mauler R, Gruschkau H, Lehmann HG. Reactogenicity to primary and repeated vaccination with influenza split virus vaccine. Dev Biol Stand 1977;39:289–93.
[25] Edelman K, Malmström K, He Q, Savolainen J, Terho EO, Mertsola J. Local reactions and IgE antibodies to pertussis toxin after acellular diphtheria-tetanus-pertussis immunization. Eur J Pediatr 1999;158:989–94.
[26] Falsey AR, Treanor JJ, Tornieporth N, Capellan J, Gorse GJ. Randomized, double-blind controlled phase 3 trial comparing the immunogenicity of high-dose and standard-dose influenza vaccine in adults 65 years of age and older. J Infect Dis 2009;200:172–80.

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RESEARCH PAPER

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Estimating rotavirus vaccine effectiveness in Japan using a screening method

Kaoru Araki[a,b], Megumi Hara[a], Yuta Sakanishi[c], Chisato Shimanoe[a], Yuichiro Nishida[a], Muneaki Matsuo[b], and Keitaro Tanaka[a]

[a]Department of Preventive Medicine, Saga University, Saga, Japan; [b]Department of Pediatrics, Saga University, Saga, Japan; [c]Community Medical Support Institute, Saga University, Saga, Japan

ABSTRACT

Rotavirus gastroenteritis is a highly contagious, acute viral disease that imposes a significant health burden worldwide. In Japan, rotavirus vaccines have been commercially available since 2011 for voluntary vaccination, but vaccine coverage and effectiveness have not been evaluated. In the absence of a vaccination registry in Japan, vaccination coverage in the general population was estimated according to the number of vaccines supplied by the manufacturer, the number of children who received financial support for vaccination, and the size of the target population. Patients with rotavirus gastroenteritis were identified by reviewing the medical records of all children who consulted 6 major hospitals in Saga Prefecture with gastroenteritis symptoms. Vaccination status among these patients was investigated by reviewing their medical records or interviewing their guardians by telephone. Vaccine effectiveness was determined using a screening method. Vaccination coverage increased with time, and it was 2-times higher in municipalities where the vaccination fee was supported. In the 2012/13 season, vaccination coverage in Saga Prefecture was 14.9% whereas the proportion of patients vaccinated was 5.1% among those with clinically diagnosed rotavirus gastroenteritis and 1.9% among those hospitalized for rotavirus gastroenteritis. Thus, vaccine effectiveness was estimated as 69.5% and 88.8%, respectively. This is the first study to evaluate rotavirus vaccination coverage and effectiveness in Japan since vaccination began.

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rotavirus gastroenteritis; rotavirus vaccine; rotavirus; screening method; vaccine effectiveness

Introduction

Rotavirus is the predominant cause of severe gastroenteritis among infants and young children, with most infections occurring from winter to early spring. Almost every child has been infected with rotavirus at least once by the age of 5 years, with subsequent infections becoming less severe because of increasing immunity. Severe dehydration resulting from rotavirus-induced diarrhea and vomiting can be fatal. In 2008, approximately 453,000 infants or children, mainly in developing countries, were estimated to have died from rotavirus gastroenteritis (RVGE).[1]

Although death resulting from rotavirus infection is rare in developed countries, it has been reported that approximately 40–50% of hospitalizations due to infectious gastroenteritis of infants and young children (<5 y of age) are caused by rotavirus.[2] Infants of less than 11 months with RVGE often require intravenous hydration and hospitalization.[3] Moreover, severe complications such as encephalopathy,[4] myocarditis,[5] and sudden unexpected death[6] have been reported.

In Japan, a study estimated that approximately 790,000 children aged 6 y or younger visited pediatric outpatient departments because of RVGE in a year.[7] Furthermore, limited studies indicate an estimated 26,500–78,000 people a year are hospitalized because of RVGE.[8-10] These estimates indicate the substantial health burden that rotavirus also presents in developed countries.

To date, 2 live oral vaccines, a monovalent human rotavirus vaccine (RV1, Rotarix®, GlaxoSmithKline Biologicals) and a pentavalent bovine-human reassortant vaccine (RV5, Rotateq®, Merck & Co., Inc.), have been licensed in more than 100 countries. These vaccines were recommended for global use by the World Health Organization in 2009 and had been integrated into national immunization programs in more than 50 countries by April, 2014.[11] After approval, both vaccines showed high effectiveness and safety for RVGE.

According to a systematic review, the effectiveness of vaccines RV1 and RV5 against severe RVGE was 94–71% and 83–75%, respectively, in high-income countries.[12] In Japan, RV1 and RV5 have been commercially available since November 2011 and July 2012, respectively. In large clinical trials, high efficacies against severe RVGE including hospitalization were shown (79–96%).[13,14]

An observational study examining changes in disease burden over time, including seasons before and after the introduction of these vaccines, has been reported in Japan.[15] In Shibata, Niigata Prefecture, Japan, the incidence rates of severe RVGE among children aged less than 3 y were found to be reduced by 71.2%, 47.7%, and 81.1% for 2012, 2013, and 2014 compared with that in 2011 before the vaccine was introduced.[15]

Although data regarding the efficacy of the vaccine in clinical trials and the impact of vaccination via an observational study have been reported in Japan, no studies assessing the

CONTACT Kaoru Araki abekaoruko1127@yahoo.co.jp

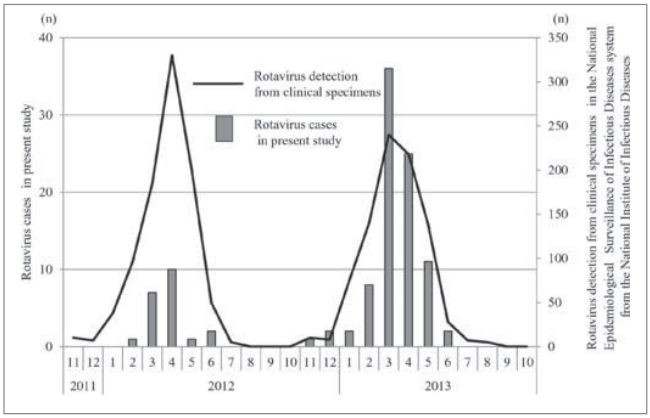

Figure 1. Monthly numbers of rotavirus cases in the 2011/12 and 2012/13 seasons (Bar graph) and those of rotavirus detection from clinical specimens, November 2011-October 2013 (Line graph).> The number of rotaviruses detected from clinical specimens was obtained from the Infectious Agents Surveillance Report.

current effectiveness of these vaccines have been published. To promote rotavirus vaccination, it is first important to establish the effectiveness of this vaccine since its introduction into Japan. The aim of this study was to investigate retrospectively changes in vaccine coverage and effectiveness since 2011 in Japan using a screening method.

Results

During the 2011/12 and 2012/13 seasons, 38 and 187 children, respectively, were consulted as part of a study of acute gastroenteritis patients. For this target group, the outbreak of disease peaked from March to April. According to the National Epidemiological Surveillance of Infectious Diseases system from the National Institute of Infectious Diseases, this trend correlated with the rotavirus outbreak data reported by a national infection research institute.[16]

As shown in Figure 1, during the 2011/12 season, target patients were limited and no patients with RVGE who had been vaccinated presented. Thus, vaccine effectiveness in the 2011/12 season could not be estimated. In the 2012/13 season, 87 children (46.5%) were diagnosed with RVGE using an immunochromatography kit. Table 1 shows the characteristics of acute gastroenteritis patients in the 2012/13 season: the median age was 11 months (age range: 2–21 months), 109 children (58.3%) were male, and 120 children (64.2%) were inpatients. The median age of the rotavirus positive cases was 13 months with 36 cases (41.4%) being children under 1 y of age. In rotavirus positive cases, severe outcomes requiring intravenous rehydration and hospitalization were more prevalent than in the rotavirus negative cases.

Figure 2 shows the change in monthly vaccine coverage following introduction of the vaccine for Saga Prefecture, Saga city, and Ogi city. In all areas, vaccine coverage increased with time (P for trend <0.01). Compared with Saga city, vaccine coverage in Ogi city, which supports the vaccination fee, was approximately 2-times higher and increased more sharply. The vaccine coverage in Saga prefecture during the 2012/13 season was 14.9%.

Table 2 shows vaccine coverage in rotavirus-positive cases and in the general population and the effectiveness of vaccination, as determined by a screening method, in the 2012/13 season. Among 87 rotavirus-positive cases, one case (1.1%) was medically examined the day after vaccination and 7 cases (8%) had an unknown vaccination status; these cases were excluded from the analysis. Four children among the remaining 79 cases received vaccine RV1. They were vaccinated with 2 doses (as recommended), and symptoms, such as vomiting and diarrhea, developed at least 1 month after the day of the last inoculation. Vaccine coverage during the 2012/13 season was estimated to be 5.1%. Vaccine coverage for rotavirus-positive cases within 12 months was 6.1% and for more than 12 months was 4.3%. According to these results, the effectiveness of vaccination for preventing RVGE in Saga for the 2012/13 season was estimated to be 69.5% (95% confidence interval [CI]: 37.1–98.9%). Vaccine effectiveness within 12 months was 71.7%, compared with 55.4% for more than 12 months. Additionally, the effectiveness in terms of reducing hospitalization owing to RVGE was estimated to be 88.8% (95% CI: 34.3–100.0%).

Table 1. Baseline characteristics of cases in the 2012/13 season.

	All cases (n = 187)		Rotavirus-positive cases (n = 87)		Rotavirus-negative cases (n = 100)		P value[a]
Hospital							<0.01
Saga University Hospital	33	(17.6)	17	(19.5)	16	(16.0)	
Saga-Ken Medical Centre Koseikan	43	(23.0)	11	(12.6)	32	(32.0)	
Saga National Hospital	29	(15.5)	11	(12.6)	18	(18.0)	
Saga Chubu Hospital	13	(7.0)	8	(9.2)	5	(5.0)	
Ureshino Medical Center	39	(20.9)	28	(32.2)	11	(11.0)	
Higashisaga Hospital	30	(16.0)	12	(13.8)	18	(18.0)	
Age, months	11	[2–21]	13	[2–21]	9	[2–20]	<0.01
Age 2–11 months	107	(57.2)	36	(41.4)	71	(71.0)	<0.01
Age 12–21 months	80	(42.8)	51	(58.6)	29	(29.0)	
Sex, male	109	(58.3)	47	(54.0)	62	(62.0)	0.27
Outcome							0.02
Outpatient (internal medicine)	58	(31.0)	23	(26.4)	35	(35.0)	
Outpatient (intravenous rehydration)	9	(4.8)	8	(9.2)	1	(1.0)	
Admission	120	(64.2)	56	(64.4)	64	(64.0)	

Data are number (percentage) or median [range].
[a]Based on chi-square test (for categorical variables) or Wilcoxon rank sum test (for continuous variables).

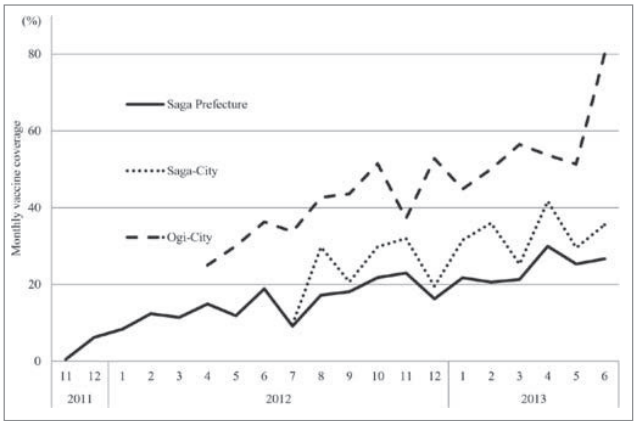

Figure 2. Monthly rotavirus vaccine coverage after vaccine introduction in Saga Prefecture (solid line), Saga city (dotted line) and Ogi city (dashed line). In all areas, vaccine coverage increased with time (P for trend <0.01).

Discussion

This is the first study to investigate the effectiveness of the rotavirus vaccine during the first 2 y after it was introduced into Japan. The effectiveness of the vaccine in protecting against RVGE, as determined by the screening method, was estimated to be 69.5%. This level of efficacy was similar to that reported by a previous clinical trial (75–79% for RVGE, 96% for hospitalization owing to RVGE),[13,14] confirming the effectiveness of the rotavirus vaccine in Japan.

Most analyses of rotavirus vaccine effectiveness following its introduction have been case-control studies, reporting rates of 80%–90% effectiveness. According to reports from developed countries (Spain,[17,18] Germany,[19] Taiwan,[20] Portugal,[21] the United States of America,[22] and Belgium[23]), the effectiveness of the vaccine against severe RVGE requiring hospitalization was higher than that against cases not requiring hospitalization. An investigation in 2010 and 2011 in the United States of America, where the rotavirus vaccine was being used routinely, reported that the effectiveness of the vaccine against hospitalization owing to RVGE was 91% (95% CI: 80%–95%).[22] Similarly, in Taiwan, where rotavirus vaccination was voluntary, the effectiveness against severe RVGE was 90.4% (95% CI: 70.3–98.1%).[20] The effectiveness of the vaccine against hospital visits owing to RVGE was 83.7% (95% CI: 73.9%–89.8%) in Portugal[21] and 83.5% (95% CI: 45.5%–99.7%) in Spain.[18] Although, in our study, vaccine effectiveness against the total number of RVGE cases was estimated to be slightly lower compared with the results of previous clinical trials,[13,14] the effectiveness of the vaccine against hospitalization for RVGE was found to be almost the same.

The current study was conducted using a screening method. Vaccine effectiveness determined by this method was similar to the findings from case-control studies and will be helpful in verifying estimates in clinical trials. In Nicaragua, 2 case-control studies for rotavirus effectiveness were conducted.[24,25] The first study was conducted by the United States Centers for Disease Control and Prevention from 2007 to 2008. The vaccine effectiveness in 2008 against severe RVGE in children aged 8 to 11 months was 69% (95% CI: 24%–87%).[24] The second study was coordinated by the Ministry of Health in Nicaragua and Merck and Co., Inc. from 2007 to 2009. Using the data from 2008, the vaccine effectiveness to prevent severe RVGE was 92% (95% CI: 79%–97%) for cases <12 months of age.[25] Cardellino.A et al employed the screening method using these data in 2008 and estimated that vaccine effectiveness was 72% (95% CI: 62%–83%) to 92% (95% CI: 78%–100%). The results were relatively consistent with those of their 2 cases-control studies.[26] The screening method is therefore reliably consistent with the data from case-control studies.

The effectiveness of the vaccine among 12–22 months was lower than that among under 12 months, indicating the possibility that the effect of the vaccine lessened with time post-vaccination. However, the present study was conducted just after the introduction of the vaccine and the proportion of the population vaccinated (PPV) after 1 y was low; therefore, the effectiveness of the vaccine may have been underestimated. There are few reports about the effect of lasting immunity after rotavirus vaccination, but a follow-up survey of RV1 in high income countries of Asia reported that the protective effects against severe cases lasted at least 3 y after vaccination.[27]

Table 2. Proportion of cases vaccinated and estimates of vaccine effectiveness in the 2012/13 season.

	Case Vaccination Status				Population Vaccination Status				
	Vaccinated (n)	Unvaccinated (n)	PCV (%)	(95% CI)	Vaccinated[a] (person-months)	Unvaccinated[a] (person-months)	PPV (%)	VE (%)	(95% CI)
Rotavirus-positive cases (n = 79)	4[b]	75	5.1[c]	(1.4–12.5)	12195.8	69814.2	14.9	69.5[c]	(37.1–98.9)
Age									
Age 2–11 months (n = 33)	2	31	6.1[c]	(0.7–20.2)	9187.5	40327.5	20.1	71.7	(−11.1–99.6)
Age 12–21 months (n = 46)	2	44	4.3[c]	(0.5–4.8)	3008.3	29486.7	9.3	55.4	(−70.3–99.0)
Outcome									
Outpatient (internal medicine) (n = 19)	2	17	10.5[c]	(1.3–33.1)	12195.8	69814.2	14.9	32.7	(−183.2–99.4)
Outpatient (intravenous rehydration) (n = 8)	1	7	12.5[c]	(0.3–52.7)	12195.8	69814.2	14.9	18.2	(−537.8–100.0)
Admission (n = 52)	1	51	1.9[c]	(0.1–10.3)	12195.8	69814.2	14.9	88.8[c]	(34.3–100.0)

CI, confidence interval. PCV, proportion of cases vaccinated. PPV, proportion of the population vaccinated. VE, vaccine effectiveness.
[a]This figure represents person-months accumulated in the corresponding category by vaccination status (and age category [2–11 or 12–21 months] in age-stratified analysis) for 8 months in the 2012/13 season. The number of children who contributed to person-months was 2032 for the vaccinated and 10281 for the unvaccinated. For details, see the "Statistical analysis".
[b]All children received vaccine RV1 with 2 doses (fully vaccinated).
[c]$P < 0.05$

Interestingly, the rise in vaccination coverage since approval of the rotavirus vaccine was higher in areas where the vaccination fee was supported. It has previously been reported that financial assistance and information are necessary to motivate parents to voluntarily vaccinate their children in Japan.[28] It is possible that in areas in which financial support for vaccination is available, more information is publicized regarding the vaccine, both of which may contribute to the observed rise in vaccine coverage.

This study had some limitations. First, vaccine coverage was calculated by the number of births and the number of shipments of the vaccine; therefore, the PPV is an estimate. This estimation does not take into account infants who died after birth, those who were unable to be vaccinated because of underlying diseases, and those who only received one dose of the vaccine and were therefore not fully protected. In April, 2013, the Ministry of Health, Labour and Welfare published rotavirus vaccine coverage data by prefecture. Vaccine coverage in Saga Prefecture was 28%, which was in accordance with our findings for the same period, 29.9%. Second, using the screening method, estimations of vaccine effectiveness may fluctuate when the PPV and the proportion of cases vaccinated (PCV) are too low.[29] In the current study, vaccine coverage was estimated just after the introduction of the vaccine and would therefore include a period of time when vaccine coverage would be low. Because this variability in the PPV would be high, vaccine effectiveness may consequently be underestimated. Additionally, because this study was based on data from one prefecture, the limited population size may confer a degree of error in the PCV. Third, in this study the target hospitals were limited to only higher order medical institutions having hospitalization facilities and emergency lifesaving centers. The effectiveness of the vaccine against mild RVGE could therefore not be estimated because almost all of the children with initial symptoms of this disease would have presented at primary facilities. However, the primary purpose of the rotavirus vaccine is to prevent hospitalization and death caused by serious vomiting and diarrhea. The effectiveness in these situations was evaluated in this study. Finally, the screening method is a relatively simple and effective method for determining vaccine effectiveness, but does not take into account confounding factors such as incomplete vaccination or other underlying diseases. It is now necessary to confirm the authenticity of our data using a more elaborate method such as a case-control study.

In conclusion, this is the first study to evaluate the effectiveness of the rotavirus vaccine by the screening method in Japan. Vaccine coverage increased with time following introduction of the vaccine and the effectiveness of the vaccine was estimated to be 69.5% for clinically diagnosed RVGE and 88.8% for hospitalized RVGE cases. These results support promotion of the rotavirus vaccination program in Japan.

Patients and methods

Investigation area

The investigation was conducted in Saga Prefecture in Japan. This area has a stable population of approximately 850,000 inhabitants, and approximately 7,500 babies are born each year. Rotavirus vaccine is voluntary in Japan and costs 13,000–15,000 Japanese yen per inoculation. Among municipalities in this area, only Ogi city has public money to assist with the vaccine program, contributing 5,000 yen per inoculation. We requested the cooperation of 6 major hospitals with pediatric outpatient departments and hospitalization facilities in Saga (Saga University Hospital, Saga-Ken Medical Centre Koseikan, Saga National Hospital, Saga Chubu Hospital, Ureshino Medical Center, and Higashisaga Hospital). These are higher order medical institutions treating the seriously ill including those with acute gastroenteritis. The survey protocol was approved by the Ethical Committee of Saga University Faculty of Medicine, Saga-Ken Medical Centre Koseikan, and Saga National Hospital. Other hospitals were approved as cooperation facilities of Saga University Faculty of Medicine.

Rotavirus gastroenteritis patients and vaccine coverage

A case was defined as any child who was born between August 1, 2011 and April 30, 2013 (able to receive the rotavirus vaccine), displayed the clinical characteristics of gastroenteritis such as vomiting and diarrhea, and who tested positive for fecal rotavirus antigen using an immunochromatography kit. The investigation period included 2 seasons between November, 2011 and June, 2012 (2011/12 season), and November, 2012 and June, 2013 (2012/13 season), when an outbreak of rotavirus occurred according to the National Epidemiological Surveillance of Infectious Diseases system from the National Institute of Infectious Diseases.[16] Vaccination information, including whether a child had received the rotavirus vaccine, the type of vaccine, the number of doses, the date of the last dose, and the outcome (oral medication, intravenous rehydration to correct dehydration, admission), was usually obtained from their medical records in the hospital in cooperation with their pediatrician. If the vaccination status was not available in their medical record, it was obtained by telephone interview with their guardian. Children administered the last dose of vaccine within 14 d before the onset of gastroenteritis were excluded because of development of a protective immune response.

Vaccine coverage of the general population

Because there is no register for national vaccination in Japan, data regarding rotavirus vaccine coverage in the general population are not readily available. We therefore obtained the number of rotavirus vaccines shipped monthly by pharmaceutical companies (GlaxoSmithKline and Merck & Co.) into Saga Prefecture and divided this by the number of doses (RV1: 2, RV5: 3). Thus, we estimated the number of people that received the vaccine in each month. This was then divided by the number of monthly births taken from the demographic data for Saga Prefecture and the cities of Saga and Ogi. In this way, we estimated the monthly vaccine coverage, as shown in Figure 2. Additionally, we obtained information for Ogi city regarding financial assistance for the vaccine.

Statistical analysis

The SAS statistical software package (Ver. 9.3 for Windows; SAS Institute, Cary, NC, USA) and Microsoft Excel (version 2010, Microsoft Japan) were used for statistical analysis. To compare the characteristics of gastroenteritis cases between rotavirus-positive cases and those negative cases, we used the χ^2 test for categorical variables and the Wilcoxon rank sum test for continuous variables. The trend of monthly increase of vaccine coverage was tested by a logistic regression model, using PROC LOGISTIC in SAS.

The effectiveness of the rotavirus vaccine was estimated by the screening method using the Farrington algorithm, according to the following formula.[30]

$$\text{Vaccine effectiveness} = 1 - \frac{PCV}{1-PCV} \times \frac{1-PPV}{PPV} = (\text{PPV} - \text{PCV}) \,/\, \text{PPV}(1 - \text{PCV});$$

PCV is the proportion of cases vaccinated, which means the proportion of vaccinated among rotavirus-positive cases. The exact 95% confidence interval (CI) for the PCV was constructed by using the BINOMIAL option in the EXACT statement of PROC FREQ in SAS. PPV is the proportion of the population vaccinated, which means vaccination coverage of the general population. We employed the person-time method to derive PPV (i.e., person-time during the investigation period in the vaccinated divided by that in both the vaccinated and unvaccinated) because the target population aged over time. Person-months accumulated in each stratum by vaccination status (and age category [2–11 or 12–21 months] in age-stratified analysis) in the target population was calculated for the 8 month period in the 2012/13 season. For example, in age-stratified analysis (Table 2), a child who was born in May 2012 contributed 6 person-months to the age category of 2–11 months and 2 person-months to that of 12–21 months, for the above 8 month period. A P value of less than 0.05 was considered statistically significant, and for vaccine effectiveness, its estimate was regarded as significant if its 95% CI did not include the null value (0).

Abbreviations

RVGE	rotavirus gastroenteritis
RV1	Rotarix®
RV5	RotaTeq®
PCV	the proportion of cases vaccinated
PPV	the proportion of the population vaccinated

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

[1] Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, the WHO-coordinated Global Rotavirus Surveilance Network. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis 2012; 12: 136-41; PMID:22030330; http://dx.doi.org/10.1016/S1473-3099(11)70253-5

[2] Parashar UD, Gibson CJ, Bresee JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerg Infect Dis 2006; 12: 304-6; PMID:16494759; http://dx.doi.org/10.3201/eid1202.050006

[3] Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003; 9: 565-72; PMID:12737740; http://dx.doi.org/10.3201/eid0905.020562

[4] Kawamura Y, Ohashi M, Ihira M, Hashimoto S, Taniguchi K, Yoshikawa T. Nationwide survey of rotavirus-associated encephalopathy and sudden unexpected death in Japan. Brain Dev 2014; 36: 601-7; PMID:23972382; http://dx.doi.org/10.1016/j.braindev.2013.07.013

[5] Grech V, Calvagna V, Falzon A, Mifsud A. Fatal, rotavirus-associated myocarditis and pneumonitis in a 2-year-old boy. Ann Trop Paediatr 2001; 21: 147-8; PMID:11471258; http://dx.doi.org/10.1080/02724930120058214

[6] Nakano I, Taniguchi K, Ishibashi-Ueda H, Maeno Y, Yamamoto N, Yui A, Komoto S, Wakata Y, Matsubara T, Ozaki N. Sudden death from systemic rotavirus infection and detection of nonstructural rotavirus proteins. J Clin Microbiol 2011; 49: 4382-5; PMID:21998424; http://dx.doi.org/10.1128/JCM.01303-11

[7] Yokoo M, Arisawa K, Nakagomi O. Estimation of annual incidence, age-specific incidence rate, and cumulative risk of rotavirus gastroenteritis among children in Japan. Jpn J Infect Dis 2004; 57: 166-71; PMID:15329449

[8] Nakagomi T, Nakagomi O, Takahashi Y, Enoki M, Suzuki T, Kilgore PE. Incidence and burden of rotavirus gastroenteritis in Japan, as estimated from a prospective sentinel hospital study. J Infect Dis 2005; 192 Suppl 1: S106-10; PMID:16088792; http://dx.doi.org/10.1086/431503

[9] Kamiya H, Nakano T, Inoue M, Abd TT, Patel M, Orenstein WA, Parashar UD. A retrospective evaluation of hospitalizations for acute gastroenteritis at 2 sentinel hospitals in central Japan to estimate the health burden of rotavirus. J Infect Dis 2009; 200 Suppl 1: S140-6; PMID:19817592; http://dx.doi.org/10.1086/605028

[10] Ito H, Otabe O, Katsumi Y, Matsui F, Kidowaki S, Mibayashi A, Nakagomi T, Nakagomi O. The incidence and direct medical cost of hospitalization due to rotavirus gastroenteritis in Kyoto, Japan, as estimated from a retrospective hospital study. Vaccine 2011; 29: 7807-10; PMID:21821087; http://dx.doi.org/10.1016/j.vaccine.2011.07.105

[11] Yen C, Tate JE, Hyde TB, Cortese MM, Lopman BA, Jiang B, Glass RI, Parashar UD. Rotavirus vaccines: current status and future considerations. Hum Vaccin Immunother 2014; 10: 1436-48; PMID:24755452; http://dx.doi.org/10.4161/hv.28857

[12] Leshem E, Lopman B, Glass R, Gentsch J, Bányai K, Parashar U, Patel M. Distribution of rotavirus strains and strain-specific effectiveness of the rotavirus vaccine after its introduction: a systematic review and meta-analysis. Lancet Infect Dis 2014; 14: 847-56; PMID:25082561; http://dx.doi.org/10.1016/S1473-3099(14)70832-1

[13] Kawamura N, Tokoeda Y, Oshima M, Okahata H, Tsutsumi H, Van Doorn LJ, Muto H, Smolenov I, Suryakiran P.V., Han HH. Efficacy, safety and immunogenicity of RIX4414 in Japanese infants during the first two years of life. Vaccine 2011; 29: 6335-41; PMID:21640780; http://dx.doi.org/10.1016/j.vaccine.2011.05.017

[14] Iwata S, Nakata S, Ukae S, Koizumi Y, Morita Y, Kuroki H, Tanaka Y, Shizuya T, Schidel F, Brown MV, Lawrence J. Efficacy and safety of pentavalent rotavirus vaccine in Japan: a randomized, double-blind, placebo-controlled, multicenter trial. Hum Vaccin Immunother 2013; 9: 1626-33; PMID:23732903; http://dx.doi.org/10.4161/hv.24846

[15] Oishi T, Taguchi T, Nakano T, Sudo S, Kuwajima H, Group SRS. The occurrence of severe rotavirus gastroenteritis in children under 3 years of age before and after the introduction of rotavirus vaccine: a prospective observational study in three pediatric clinics in Shibata City, Niigata Prefecture, Japan. Jpn J Infect Dis 2014; 67: 304-6; PMID:25056079; http://dx.doi.org/10.7883/yoken.67.304

[16] National Institute of Ingectious Diseases and Tuberculosis asnd Infectious Diseases Control Division MoH, Labour and Welfare. Rotavirus,2010-2013,Japan. Japan: Infectious Agents Surveillance Report, 2014.

[17] Castilla J, Beristain X, Martínez-Artola V, Navascués A, García Cenoz M, Alvarez N, Polo I, Mazon A, Gil-setas A, Barricarte A. Effectiveness of rotavirus vaccines in preventing cases and hospitalizations due to rotavirus gastroenteritis in Navarre, Spain. Vaccine 2012; 30: 539-43; PMID:22122860; http://dx.doi.org/10.1016/j.vaccine.2011.11.071

[18] Bellido-Blasco JB, Sabater-Vidal S, Salvador-Ribera MeM, Arnedo-Pena A, Tirado-Balaguer MD, Meseguer-Ferrer N, Silvestre-Silvestre E, Romeu-Garcia MA, Herrero-Carot C, Moreno-Munoz M-R. Rotavirus vaccination effectiveness: a case-case study in the EDICS project, Castellón (Spain). Vaccine 2012; 30: 7536-40; PMID:23103196; http://dx.doi.org/10.1016/j.vaccine.2012.10.049

[19] Adlhoch C, Hoehne M, Littmann M, Marques AM, Lerche A, Dehnert M, Eckmanns T, Wichimann O, Koch J. Rotavirus vaccine effectiveness and case-control study on risk factors for breakthrough infections in Germany, 2010-2011. Pediatr Infect Dis J 2013; 32: e82-9; PMID:23334342; http://dx.doi.org/10.1097/INF.0b013e3182720b71

[20] Chang WC, Yen C, Wu FT, Huang YC, Lin JS, Huang FC, Yu HT, Chi CL, Lin HY, Tate JE, Parashar UD, Wu HS, Hsiung CA. Effectiveness of 2 rotavirus vaccines against rotavirus disease in Taiwanese infants. Pediatr Infect Dis J 2014; 33: e81-6; PMID:24569388; http://dx.doi.org/10.1097/INF.0000000000000105

[21] Marlow R, Ferreira M, Cordeiro E, Trotter C, Januário L, Finn A, Rodrigues F. Case control study of rotavirus vaccine effectiveness in portugal during six years of private market use. Pediatr Infect Dis J 2014

[22] Cortese MM, Immergluck LC, Held M, Jain S, Chan T, Grizas AP, Knizew S, Barrett C, Quaye O, Mijatovic-Rustempasic S, et al. Effectiveness of monovalent and pentavalent rotavirus vaccine. Pediatrics 2013; 132: e25-33; PMID:23776114; http://dx.doi.org/10.1542/peds.2012-3804

[23] Braeckman T, Van Herck K, Meyer N, Pirçon JY, Soriano-Gabarró M, Heylen E, Zeller M, Azou M, Capiau H, Koster JD, et al. Effectiveness of rotavirus vaccination in prevention of hospital admissions for rotavirus gastroenteritis among young children in Belgium: case-control study. BMJ 2012; 345: e4752; PMID:22875947

[24] Patel M, Pedreira C, De Oliveira LH, Tate J, Orozco M, Mercado J, Gonzalez A, Malespin O, Amador J J, Umana J, et al. Association between pentavalent rotavirus vaccine and severe rotavirus diarrhea among children in Nicaragua. JAMA 2009; 301: 2243-51; PMID:19491186; http://dx.doi.org/10.1001/jama.2009.756

[25] Mast TC, Khawaja S, Espinoza F, Paniagua M, Del Carmen LP, Cardellino A, Sanchez E. Case-control study of the effectiveness of vaccination with pentavalent rotavirus vaccine in Nicaragua. Pediatr Infect Dis J 2011; 30: e209-15; PMID:21768920; http://dx.doi.org/10.1097/INF.0b013e31822a8527

[26] Cardellino A, Khawaja S, Sánchez Cruz E, Mast TC. Effectiveness of vaccination with the pentavalent rotavirus vaccine in Nicaragua as determined using the screening method. Hum Vaccin Immunother 2013; 9: 1449-53; PMID:23571175; http://dx.doi.org/10.4161/hv.24338

[27] Phua KB, Lim FS, Lau YL, Nelson EA, Huang LM, Quak SH, Lee BW, Doorn LJ, Teoh YL, Teoh YL, et al. Rotavirus vaccine RIX4414 efficacy sustained during the third year of life: a randomized clinical trial in an Asian population. Vaccine 2012; 30: 4552-7; PMID:22497874; http://dx.doi.org/10.1016/j.vaccine.2012.03.030

[28] Shono A, Kondo M. Factors that affect voluntary vaccination of children in Japan. Vaccine 2014; 33: 1406-11; PMID:25529291; http://dx.doi.org/10.1016/j.vaccine.2014.12.014

[29] Chen RT, Orenstein WA. Epidemiologic methods in immunization programs. Epidemiol Rev 1996; 18: 99-117; PMID:9021306; http://dx.doi.org/10.1093/oxfordjournals.epirev.a017931

[30] Farrington CP. Estimation of vaccine effectiveness using the screening method. Int J Epidemiol 1993; 22: 742-6; PMID:8225751; http://dx.doi.org/10.1093/ije/22.4.742

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Original Article

Immunogenicity and Efficacy of A/H1N1pdm Vaccine Among Subjects With Severe Motor and Intellectual Disability in the 2010/11 Influenza Season

Megumi Hara[1], Tomoyuki Hanaoka[2,3,4], Kazuhiro Maeda[5], Tetsuo Kase[6], Satoko Ohfuji[7], Wakaba Fukushima[7], and Yoshio Hirota[7,8,9]

[1]Department of Preventive Medicine, Faculty of Medicine, Saga University, Saga, Japan
[2]Bihoro Ryoiku Hospital, Abashiri, Hokkaido, Japan
[3]Department of Hygiene and Preventive Medicine, Showa University, School of Medicine, Tokyo, Japan
[4]Hokkaido University Center for Environmental and Health Sciences, Sapporo, Japan
[5]The Research Foundation for Microbial Diseases of Osaka University, Kannonji, Kagawa, Japan
[6]Osaka Prefectural Institute of Public Health, Osaka, Japan
[7]Department of Public Health, Faculty of Medicine, Osaka City University, Osaka, Japan
[8]Clinical Epidemiology Research Center, Medical Co. LTA, Fukuoka, Japan
[9]College of Healthcare Management, Fukuoka, Japan

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ABSTRACT

Background: While the immunogenicity and effectiveness of seasonal influenza vaccines among subjects with severe motor and intellectual disability (SMID) are known to be diminished, the efficacy of the A/H1N1pdm vaccine has not been evaluated.

Methods: We prospectively evaluated 103 subjects with SMID (mean age, 41.7 years) who received trivalent inactivated influenza vaccine during the 2010/11 influenza season. The hemagglutination inhibition (HI) antibody titer was measured in serum samples collected pre-vaccination (S0), post-vaccination (S1), and end-of-season (S2) to evaluate subjects' immunogenicity capacity. Vaccine efficacy was assessed based on antibody efficacy and achievement proportion.

Results: The proportions of seroprotection and seroconversion, and the geometric mean titer (GMT) ratio (GMT at S1/GMT at S0) for A/H1N1pdm were 46.0%, 16.0%, and 1.8, respectively—values which did not meet the European Medicines Evaluation Agency criteria. The achievement proportion was 26%. During follow-up, 11 of 43 subjects with acute respiratory illness were diagnosed with type A influenza according to a rapid influenza diagnostic test (RIDT), and A/H1N1pdm strains were isolated from the throat swabs of 5 of those 11 subjects. When either or both RIDT-diagnosed influenza or serologically diagnosed influenza (HI titer at S2/HI titer at S1 $\geq$2) were defined as probable influenza, subjects with A/H1N1pdm seroprotection were found to have a lower incidence of probable influenza (odds ratio, 0.31; antibody efficacy, 69%; vaccine efficacy, 18%).

Conclusions: In the present seasonal assessment, antibody efficacy was moderate against A/H1N1pdm among SMID subjects, but vaccine efficacy was low due to the reduced immunogenicity of SMID subjects.

Key words: influenza vaccine; immunogenicity; antibody efficacy; vaccine efficacy; disabled person

INTRODUCTION

Severe motor and intellectual disability (SMID) is defined as being bedridden or only able to sit, crawl, or walk with support and having a relatively low intelligence quotient (<35).[1] Further, such individuals are generally debilitated and also immunocompromised,[2] with a lower immunogenicity to seasonal influenza vaccines than healthy individuals and no booster effect; moreover, age has a greater influence on immunogenicity than on symptom severity in this population.[2,3] We previously reported that, in the 2009 A/H1N1pdm influenza pandemic, a single dose of A/H1N1pdm monovalent vaccine did not induce sufficient immunity in individuals with SMID, and a second dose was likely to be

Address for correspondence. Megumi Hara, Department of Preventive Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan (e-mail: harameg@cc.saga-u.ac.jp).

ineffective as well, given the diminished immunogenicity capacity of this population.[4] Because pre-vaccination levels of hemagglutination inhibition (HI) antibodies against the vaccine strain significantly influence immunogenicity,[5] studies to determine whether or not immunogenicity improved in subjects with SMID in the following season are required.

Similar to immunogenicity, vaccine efficacy is also suspected to be diminished among subjects with SMID. However, vaccine efficacy is based on the percentage reduction in incidence of influenza in vaccinated subjects compared to that in unvaccinated subjects.[6,7] Given that some 40%–60% of SMID subjects reside in chronic-care facilities in Japan[1] and most are vaccinated, evaluating vaccine efficacy is difficult.

In such situations, an index of "antibody efficacy"[8] is used, which was described by Longini et al in 1988 and reflects the percentage reduction in influenza incidence among subjects with a protective post-vaccination HI titer compared with that among subjects without this HI titer. Antibody efficacy has two important advantages over vaccine efficacy: it can estimate vaccine efficacy in any target population, even a population with 100% vaccination coverage, and it can do so blindly. Because pre-vaccination, post-vaccination, and end-of-season HI titers are analyzed simultaneously at the end of follow-up, the outcomes are evaluated without knowledge of the subjects' HI titer status. An accurate determination of the incidence of strain-specific influenza is crucial to calculating antibody efficacy. However, only a few studies have used antibody efficacy to assess vaccine efficacy[9–11] because of the difficulties involved in virological confirmation.

Here, to estimate vaccine efficacy of nonadjuvanted trivalent inactivated influenza vaccine (IIV3) including the A/H1N1pdm strain in subjects with SMID, we conducted a prospective observational study evaluating the immunogenicity and antibody efficacy of this vaccine during the 2010/11 influenza season.

METHODS

Study subjects and study season

Our study was conducted in Japan during the 2010/2011 influenza season. According to reports from the Hokkaido Infectious Disease Surveillance Center, as recorded by the National Epidemiological Surveillance of Infectious Disease, an influenza epidemic occurred between January 10 and May 28, 2011 in the Abashiri area, where the research facility was located. Circulating strains were antigenically well matched to A/H1N1pdm.[12]

Study subjects were 103 individuals with SMID who mainly suffered from cerebral palsy, epilepsy, and cognitive disorders and resided in a long-term care facility in Hokkaido Prefecture, located in northern Japan. They had received 2 doses of nonadjuvanted split-virus A/H1N1pdm vaccine containing at least 15 μg hemagglutinin antigen to A/California/7/2009 (A/H1N1)v-like strain from the 2009 pandemic (lot no. HP01A in 2009; Research Foundation for Microbial Disease of Osaka University, Osaka, Japan). None of the subjects had been infected with the influenza strain from the 2009/10 season.[4]

Standard protocol approval, registration, and patient consent

The study subjects' guardians provided written informed consent for their participation in our study. The baseline characteristics of subjects, including age, sex, and chronic medical conditions, were collected from medical records. None of the subjects had a history of allergy to eggs or anaphylaxis to vaccine components. The study protocol was approved by the Institutional Review Board of the Saga University Faculty of Medicine (H21-54) and was conducted in accordance with the Ethical Guidelines for Epidemiological Research of the Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare of Japan. The study was registered in the UMIN Clinical Trials Registry (UMIN000015037).

Vaccination and serum specimen collection

All subjects received a single dose of 0.5 mL IIV3 (lot no. HA110A; Research Foundation for Microbial Disease of Osaka University) subcutaneously into their arm on November 1, 2010. The vaccine contained at least 15 μg each of hemagglutinin antigen to A/California/7/2009 (H1N1)pdm, A/Victoria/210/2009 (H3N2), and B/Brisbane/60/208. After vaccination, healthcare workers at the facility carefully observed vaccinated subjects for anaphylactic shock for at least 30 minutes and adverse reactions for 48 hours following vaccination, including either local (erythema, swelling, induration, itching, and pain) or general reactions (fever, fatigue, myalgia or arthralgia, headache, and rash).

Serum samples were collected before vaccination (S0), three weeks after vaccination (S1), and six months after vaccination (S2). All serum samples were stored at −40°C until assayed.

Measurement and evaluation for immunogenicity

The serum antibody titer against the vaccine strain was measured routinely using the HI assay with chicken erythrocytes.[13,14] Serum samples were treated with receptor-destroying enzyme (*Vibrio cholera* filtrate; Denka Seiken, Tokyo, Japan) to inactivate nonspecific inhibitors. All samples were assayed simultaneously at the laboratory of the Research Foundation for Microbial Disease of Osaka University.

The geometric mean titer (GMT) of HI, seroprotection proportion (proportion of subjects with HI titer ≥1:40 at S1), and seroconversion proportion (proportion of subjects with HI titer <1:10 at S0 and ≥1:40 S1, or ≥1:10 at S0 with a 4-fold increase in titer at S1 compared with that at S0) were calculated. If HI titers were below or above the detection

limits (<1:10 or >1:5120), they were set as 1:5 or 1:5120, respectively. The GMTs at S1 were compared with those at S0, and the GMT ratio was calculated. The achievement proportion was calculated as the proportion of subjects with an HI titer <1:40 at S0 and ≥1:40 at S1.

Immunogenicity was evaluated according to the European Medicines Evaluation Agency (EMEA) criteria for evaluating HI antibody responses to seasonal vaccine.[15] The cut-off values for vaccine immunogenicity in adults aged 18–60 years were a seroprotection proportion >70%, seroconversion proportion >40%, or mean geometric increase >2.5.

Follow-up and definition of outcome

After vaccination, all subjects were followed from the November 1, 2010 to May 31, 2011. Healthcare workers measured subjects' body temperature every morning and afternoon and prospectively recorded respiratory symptoms (cough, sore throat, and nasal congestion) and other general symptoms (fever, muscle pain, and general fatigue). When subjects had a fever (body temperature ≥37.8°C), throat swabs were collected and tested using a rapid influenza diagnosis test (RIDT; Capilia FluA, B; Becton-Dickinson Japan, Tokyo, Japan), based on an immunochromatographic method. If the test was positive for infection, throat swabs collected from the patients were stored at −40°C. To confirm the existence and strain of the influenza virus in potentially infected patients, we cultured the circulating influenza virus using standard methods at the Osaka Prefectural Institute of Public Health laboratory.

We established six outcomes for vaccine effectiveness: acute respiratory illness (ARI), defined as sudden-onset fever (body temperature ≥37.8°C)[16]; influenza-like illness (ILI), defined as ARI within the influenza epidemic period when RIDT-diagnosed influenza cases were observed (from January 17 to February 6); RIDT-diagnosed influenza; serologically diagnosed influenza 1 (HI titer at S2/HI titer at S1 ≥4); serologically diagnosed influenza 2 (HI titer at S2/HI titer at S1 ≥2); and probable influenza, defined as RIDT-diagnosed influenza and an RIDT-negative result with serologically diagnosed influenza 2.

Statistical analysis

All statistical analyses were performed using SAS 9.3 for Windows (SAS Institute, Cary, NC, USA). Regarding immunogenicity, the 95% confidence intervals (CIs) of seroprotection and seroconversion proportions were calculated using the exact binomial distribution for proportions. Wilcoxon's rank-sum test was used to compare GMT and GMT ratios between groups, while Wilcoxon's signed-rank test was used to determine the significance of the increase in HI antibody titers post-vaccination in each group. The baseline characteristics and seroprotection proportion were compared using the chi-square test or Fisher's exact test.

To clarify confounding factors for antibody efficacy, we initially examined factors associated with both seroprotection and outcomes. Odds ratios (OR) and 95% CIs of subjects exhibiting post-vaccination seroprotection for each outcome were then calculated by multiple logistic regression, with adjustment for possible confounding factors. Antibody efficacy was calculated as follows: [1 − adjusted OR] × 100%. The product of antibody efficacy and achievement proportion is theoretically equivalent to vaccine efficacy.[9]

RESULTS

A total of 103 subjects with SMID (56 men and 47 women; mean age, 41.7 [standard deviation, 10.4] years) were institutionalized with one or more of the following: cerebral palsy ($n = 45$), epilepsy ($n = 29$), intelligence impairment ($n = 8$), post-meningitis ($n = 6$), and other reasons ($n = 25$). Subjects had no influenza infection within one month or three weeks after vaccination. No serious adverse events occurred during the study period. While several mild adverse reactions, such as local redness and swelling, were reported, these were transient.

Subjects' immunogenicity to A/H1N1pdm did not meet the EMEA criteria (Table 1). The seroprotection proportion was 46%, the seroconversion proportion 16%, and the GMT ratio 1.8, with an achievement proportion of 26%. Age was not associated with immunogenicity. The GMT ratio was lower in male subjects than in female subjects, and the seroprotection proportion was significantly lower in those with asthma than in those without ($P = 0.02$). Subjects with a higher pre-vaccination HI titer had a lower GMT ratio and higher proportion of seroprotection than those with relatively low pre-vaccination titers. Subjects with a pre-vaccination HI titer of 1:10–20 had a higher seroconversion proportion and achievement proportion than those with an HI titer <1:10.

Figure shows the numbers of ARI cases and RIDT-diagnosed influenza cases in this study population during the observation period. Among 43 cases of ARI, 11 were diagnosed as type A influenza by RIDT, and of these 11, 5 were virologically confirmed to have A/H1N1pdm influenza.

Given that asthma was associated with a reduced seroprotection proportion against H1N1pdm (OR 0.11; 95% CI, 0.01–0.93) and with every outcome (eTable 1), asthma was considered a confounding factor for antibody efficacy. The crude and asthma-adjusted ORs of seroprotection, antibody efficacy, and vaccine efficacy against A/H1N1pdm for the six outcomes are summarized in Table 2. Asthma-adjusted ORs were decreased when more specific outcomes were used, with values of 0.82 (95% CI, 0.36–1.88) for ARI, 0.58 (95% CI, 0.20–1.63) for ILI, and 0.52 (95% CI, 0.12–2.24) for RIDT-diagnosed influenza. The asthma-adjusted OR of serologically diagnosed influenza 1 was lower than that of serologically diagnosed influenza 2. The asthma-adjusted OR of probable influenza was 0.31 (95% CI,

Table 1. Immunogenicity against A/H1N1pdm among subjects

	n	GMT		GMT ratio		Seroprotection	Seroconversion	Achievement	
		Pre (S0)	Post (S1)	S1/S0		*n* (%, 95% CI)	*n* (%, 95% CI)	*n*[a]	*n* (%, 95% CI)
Total	103	16	29	1.8	($P < 0.0001$)	47 (46, 36–55)	16 (16, 9–23)	73	19 (26, 16–36)
Sex									
Male	56	15	24	1.6	($P < 0.0001$)	21 (38, 25–50)	6 (11, 3–19)	41	8 (20, 9–32)
Female	47	17	36	2.2	($P < 0.0001$)	26 (55, 41–70)	10 (21, 10–33)	32	11 (34, 19–53)
		($P = 0.77$)	($P = 0.07$)	($P = 0.04$)		($P = 0.08$)	($P = 0.18$)		($P = 0.18$)
Age, years									
<30	23	19	41	2.1	($P < 0.0001$)	13 (57, 36–77)	4 (17, 2–33)	13	3 (23, 5–54)
30–39	21	18	31	1.8	($P < 0.0001$)	11 (52, 31–74)	6 (29, 9–48)	17	8 (47, 23–72)
40–49	23	13	25	1.9	($P < 0.0001$)	10 (43, 23–64)	2 (9, 0–20)	16	3 (19, 4–46)
≥50	36	15	24	1.6	($P < 0.0001$)	13 (36, 20–52)	4 (11, 1–21)	27	5 (19, 6–38)
		($P = 0.63$)	($P = 0.25$)	($P = 0.50$)		($P = 0.42$)	($P = 0.25$)		($P = 0.25$)
Asthma									
Without	93	17	30	1.8	($P < 0.0001$)	46 (49, 39–60)	16 (17, 10–25)	64	19 (30, 19–42)
With	10	9	17	2.0	($P = 0.0002$)	1 (10, 0–29)	0 (0, 0)	9	0 (0, 0–34)
		($P = 0.04$)	($P = 0.10$)	($P = 0.29$)		($P = 0.02$)	($P = 0.35$)		($P = 0.05$)
Pre-vaccination HI titer against A/H1N1									
<1:10	33	5	12	2.4	($P < 0.0001$)	2 (6, 0–14)	2 (6, 0–14)	33	2 (6, 1–20)
1:10–1:20	40	14	26	1.9	($P < 0.0001$)	17 (43, 27–58)	11 (28, 14–41)	40	17 (43, 27–59)
≥1:40	30	65	84	1.3	($P = 0.0625$)	28 (93, 84–100)	3 (10, 0–20)		($P < 0.01$)
		($P < 0.01$)	($P < 0.01$)	($P < 0.01$)		($P < 0.01$)	($P = 0.61$)		

SMIDs, severe motor and intellectual disabilities; GMT, geometric mean titer; GMTR, GMT ratio; CI, confidence interval.
Comparisons with pre-vaccination data were made using Wilcoxon's signed-rank test, and comparisons between categories were made using Wilcoxon's rank-sum test, Wilcoxon's signed-rank test, Chi-square test, or Fisher's exact test.
[a]Number of subjects with a pre-vaccination HI antibody titer <1:40.

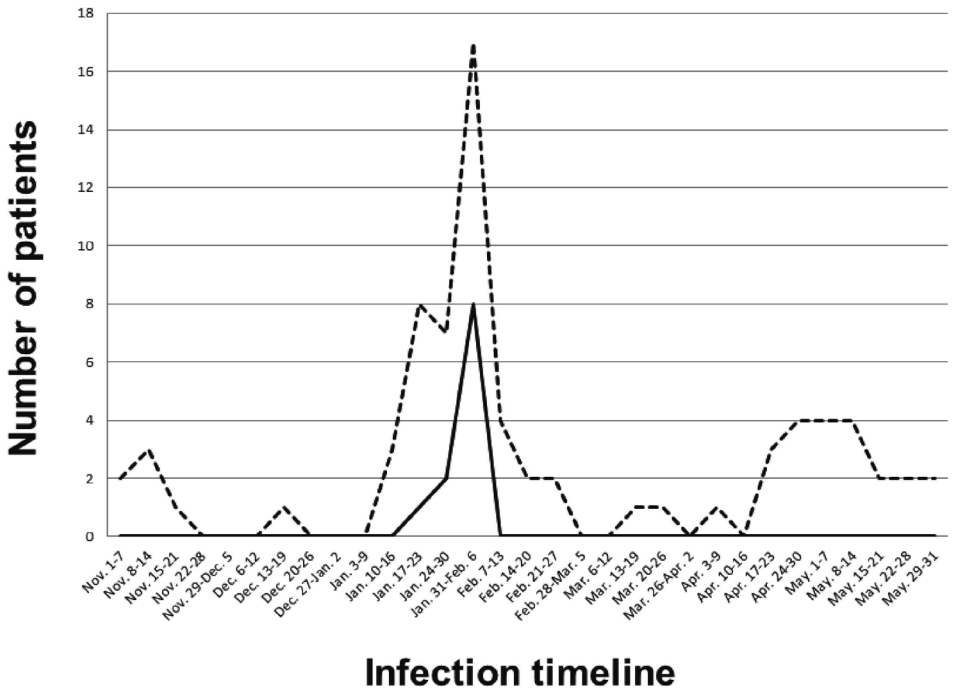

Figure. Numbers of ARI (dotted line) and RIDT-diagnosed influenza (solid line) cases during the observation period. ARI, acute respiratory illness; RIDT, rapid influenza diagnosis test.

Table 2. Effectiveness of influenza vaccine for influenza-related outcomes evaluated based on asthma-adjusted ORs, antibody efficacy, and vaccine efficacy

Outcomes	Post-vaccination		Crude OR	95% CI	Asthma-adjusted OR	95% CI	Antibody Efficacy (%)[a]	95% CI	Vaccine Efficacy (%)[b]	95% CI
	HI < 40 (n = 56)	HI ≥ 40 (n = 47)								
ARI (fever ≥37.8°C)	26	17	0.65	(0.30 to 1.45)	0.82	(0.36 to 1.88)	18.0	(−88 to 64)	4.7	(−22.9 to 16.6)
ILI (ARI within influenza endemic period)	16	7	0.44	(0.16 to 1.18)	0.58	(0.20 to 1.63)	42.4	(−63 to 80)	11.0	(−16.4 to 20.8)
RIDT-diagnosed influenza	8	3	0.41	(0.10 to 1.64)	0.52	(0.12 to 2.24)	47.6	(−124 to 88)	12.4	(−32.2 to 22.9)
Serologically diagnosed influenza 1[c]	12	2	0.16	(0.03 to 0.77)	0.20	(0.04 to 0.99)	79.8	(1 to 96)	20.7	(0.3 to 25.0)
Serologically diagnosed influenza 2[d]	14	4	0.28	(0.09 to 0.92)	0.33	(0.10 to 1.11)	67.4	(−11 to 90)	17.5	(−2.9 to 23.4)
Probable influenza[e]	12	3	0.25	(0.07 to 0.95)	0.31	(0.08 to 1.22)	69.1	(−22 to 92)	18.0	(−5.7 to 23.9)

ARI, acute respiratory illness; CI, confidence interval; HI, hemagglutination inhibition; ILI, influenza-like illness; OR, odds ratio; RIDT, rapid influenza diagnosis test; S1, post-vaccination; S2, end of the season.
[a][1 − asthma-adjusted OR] × 100%.
[b]Antibody efficacy × achievement proportion (0.26).
[c]HI titer at S2/HI titer at S1 ≥4.
[d]HI titer at S2/HI titer at S1 ≥2.
[e]RIDT-diagnosed influenza and an RIDT-negative result with serologically diagnosed influenza 2.

0.08–1.22). Given the above findings, the antibody efficacy and vaccine efficacy were determined to be 69.1% and 18.0%, respectively.

DISCUSSION

The immunogenicity of the A/H1N1pdm vaccine strain among subjects with SMID was deemed insufficient based on international standards, even after vaccination in the second season. Priming as a result of prior exposure to a related influenza strain through infection or immunization is well known to promptly induce a potent antibody response to immunization.[5] Preexisting memory T and B cells are involved in rapid and strong responses to a second vaccination, and memory T cells are crucial for controlling humoral and cellular immune responses.[17] The SMID subjects in the present study were considered to have diminished immunogenicity, given their decreased cellular immune responses and lack of a history of A/H1N1pdm influenza infection in the 2009/10 influenza season. A high pre-vaccination HI titer generally contributes to higher seroprotection, and our present findings confirmed that this association held true even among subjects with SMID. In contrast, a high pre-vaccination HI titer is generally associated with lower seroconversion proportions. However, in the present study, seroconversion proportions were the lowest in subjects with no detectable antibody levels at pre-vaccination despite receiving an A/H1N1pdm vaccine the previous influenza season. Taken together, these results indicate that non-responders to A/H1N1pdm vaccination have diminished cellular function, such as impaired immune memory or reduced immune response.

In the present study, immunogenicity was significantly diminished among subjects with a history of asthma, although details regarding their asthma treatment were unknown. Inhaled steroid hormones are usually used as preventive medication for asthma attacks, and injection or oral steroids are used for controlling attacks. Given that steroids are considered to affect T-cell immunity, steroid treatment may reduce vaccine immunogenicity.[18] Several studies have investigated the immunogenicity of inactivated influenza vaccine among asthma patients. Hanania et al reported that immune response to the A antigens of IIV3 in asthma patients was not adversely affected by inhaled corticosteroids,[19] and Bae et al reported that IIV3 induced a protective immune response in children with recurrent wheezing requiring frequent steroid treatment.[20] While these previous findings conflict with our own, Bae et al's study was conducted among children, and Hanania et al's study was conducted among children and adults. In contrast, Busse et al reported that patients aged more than 60 years with severe asthma had lower immunogenicity than younger patients with severe asthma, and their immunogenicity did not meet the EMEA criteria.[21] Further, SMIDs have been reported to be associated with rapid physical aging and degeneration.[2] Our present findings concur with those of Busse et al, as asthma was related to lower immunogenicity. In addition, asthma condition was associated with outcomes, as SMID patients with asthma had significantly higher ORs for ARI, ILI, serologically diagnosed influenza 1, and probable influenza than those without asthma (eTable 1). We therefore conducted multivariate analysis to control for its confounding effects.[22]

To our knowledge, ours is the first study to evaluate the effectiveness of IIV3 that includes A/H1N1pdm among subjects with SMIDs. Although no statistical significance was observed for most outcomes due to limited power, the ORs of seroprotection were <1 for all outcomes, suggesting relatively low influenza incidence in the population. Because misclassification of influenza generally reduces vaccine effectiveness, specific definitions for outcomes must be used to increase accuracy of influenza diagnosis.[23] When we limited ARIs to ILI or RIDT-diagnosed influenza, adjusted

ORs were decreased. Although the RIDT has high specificity and moderate sensitivity, its accuracy depends on virus count, resulting in the potential for false negatives. For instance, time since fever onset and sampling technique may influence virus count. To identify false negative cases, we combined serological diagnoses with RIDT.[24] Given that false negative RIDT results lead to misclassification of influenza-related outcomes, the effectiveness of vaccination may be underestimated.

Although serological diagnosis can be used to confirm influenza in cases with negative RIDT results, the definition of influenza infection should be considered. The adjusted OR was lowest when a four-fold increase in HI titers was used for serological diagnosis; however, this OR for four-fold increase in HI titers is considered to be an overestimation of vaccine effectiveness, because subjects with a seroprotective HI titer are unlikely to have a four-fold increase in titer after infection compared to those without a seroprotective HI titer. We therefore concluded that a two-fold increase in HI titer was a better index than a four-fold increase for confirming subclinical influenza infection in the present study. RIDT-diagnosed influenza and RIDT-negative ILI with serologically diagnosed influenza 2, which was considered a false negative result for the RIDT test, was defined as probable influenza, which was the main outcome.

Because protective HI titers represent the level at which approximately 50% of subjects will be protected,[25] a value of 69% for antibody efficacy against probable influenza might be considered beneficial. Antibody efficacy is strongly influenced by the degree of similarity between the vaccine strains and the epidemic virus. In the 2009/10 pandemic season, when vaccine and virus strains were perfectly matched—as the vaccine had been made using the circulating viral strain—antibody efficacy among pregnant women was reported as 91%.[11] In the 1991/92 season, when the virus strains were antigenically similar to vaccine strains, the antibody efficacy for A/H3N2 was 86% among healthy adults.[9] Another study reported that the antibody efficacy for A/H3N2 among institutionalized elderly individuals was 65% in the 2002/03 season, when only 42% of the A/H3N2 isolates were antigenically identical to the vaccine strain.[10] In the present study season, vaccine and circulating viral strains were well matched antigenically; therefore, antibody efficacy was expected to be higher than our findings suggest. This discrepancy may be due to the fact that influenza infection spread more easily in this population than among subjects in studies for the 2009/10 and 1991/92 seasons, as our study subjects resided in a long-term care facility. Additionally, their lower cellular and humoral immunity may have contributed to influenza infection.

The vaccine efficacy against A/H1N1pdm in the present study was estimated to be 18%, which is lower than efficacy reported in another Japanese study (47.6%)[26] and a European Union study (55%),[27] both of which were conducted using a test-negative case-control design among healthy adults in the 2010/11 season. Low immunogenicity caused low vaccine efficacy among subjects with SMID in the present study. If the achievement proportion exceeds 70%, then the vaccine efficacy is expected to exceed 48%. Improving the vaccine efficacy in SMID patients will required further studies to clarify the mechanism behind the population's low immunogenicity.

The main strength of the present study is the prospective follow-up design. We observed body temperature, respiratory symptoms, and general symptoms in each subject throughout the influenza season. This active follow-up allowed for virological confirmation of the circulating virus strain in the institution. Further, to enhance the outcome accuracy, we used a combination of RIDT and serological diagnosis, which enabled accurate estimation of antibody efficacy. However, a major limitation to the present study warrants mention. Our sample size was too small to detect statistical significance of antibody efficacy, although the analysis had a statistical power of at least 80% to detect a seroconversion proportion >40%. Our study was also single-center, so generalizability may be limited because influenza epidemics differ substantially by location, season, and population. Further, there were few other institutions in the area, limiting our population size.

In conclusion, immunogenicity against A/H1N1pdm in subjects with SMID did not improve in the second influenza season after immunization. Antibody efficacy was moderate for probable influenza among SMID subjects, but vaccine efficacy was insufficient due to the reduced immunogenicity of SMID subjects.

ONLINE ONLY MATERIAL

eTable 1. Crude ORs for each outcome with respect to subject characteristics.

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Conflicts of interest: None declared.

REFERENCES

1. Hanaoka T, Mita K, Hiramoto A, Suzuki Y, Maruyama S, Nakadate T, et al. Survival prognosis of Japanese with severe motor and intellectual disabilities living in public and private institutions between 1961 and 2003. J Epidemiol. 2010;20: 77–81.

2. Otsuka T, Fujinaka H, Katsuyama K, Iizawa M, Kinoshita S, Tanaka Y, et al. Influenza vaccination for severely multiply handicapped persons/children in the 2005–2006 season. Vaccine. 2007;25:4521–4.
3. Otsuka T, Fujinaka H, Kamimura T, Tanaka Y, Hayakawa H, Sato M, et al. Influenza vaccination in severely multiply handicapped persons/children. Vaccine. 2006;24:4096–101.
4. Hara M, Hanaoka T, Mizushima T, Honma J, Maeda K, Ohfuji S, et al. Diminished immunogenicity to pandemic H1N1 2009 influenza vaccine in subjects with severe motor and intellectual disability. Vaccine. 2011;29:8323–9.
5. Dormitzer PR, Galli G, Castellino F, Golding H, Khurana S, Del Giudice G, et al. Influenza vaccine immunology. Immunol Rev. 2011;239:167–77.
6. Halloran ME, Haber M, Longini IM Jr, Struchiner CJ. Direct and indirect effects in vaccine efficacy and effectiveness. Am J Epidemiol. 1991;133:323–31.
7. Halloran ME, Struchiner CJ, Longini IM Jr. Study designs for evaluating different efficacy and effectiveness aspects of vaccines. Am J Epidemiol. 1997;146:789–803.
8. Longini IM Jr, Koopman JS, Haber M, Cotsonis GA. Statistical inference for infectious diseases. Risk-specific household and community transmission parameters. Am J Epidemiol. 1988; 128:845–59.
9. Hirota Y, Kaji M, Ide S, Kajiwara J, Kataoka K, Goto S, et al. Antibody efficacy as a keen index to evaluate influenza vaccine effectiveness. Vaccine. 1997;15:962–7.
10. Hara M, Tanaka K, Kase T, Maeda A, Hirota Y. Evaluation of seasonal influenza vaccination effectiveness based on antibody efficacy among the institutionalized elderly in Japan. Vaccine. 2010;28:5664–8.
11. Fukushima W, Ohfuji S, Deguchi M, Kawabata K, Hatayama H, Yoshida H, et al. Effectiveness of an influenza A (H1N1) 2009 monovalent vaccine among Japanese pregnant women: a prospective observational study assessing antibody efficacy. Vaccine. 2012;30:7630–6.
12. Hokkaido Infectious Disease Surveillance Center. Surveillance data at Abashiri public health center. Available from: www.iph.pref.hokkaido.jp/kansen/abashiri/index.html. Accessed May 21, 2015.
13. Hirst GK. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. J Exp Med. 1942;75:49–64.
14. World Health Organization. WHO manual on animal influenza diagnosis and surveillance. 2002.
15. Committee for Proprietary Medical Products. Note for Guidance on Harmonisation of Requirements for Influenza Vaccines. London: European Medicines Agency; 1996. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf. Accessed May 21, 2015.
16. Hara M, Sakamoto T, Tanaka K. Effectiveness of influenza vaccination in preventing influenza-like illness among community-dwelling elderly: population-based cohort study in Japan. Vaccine. 2006;24:5546–51.
17. Weinberger B, Herndler-Brandstetter D, Schwanninger A, Weiskopf D, Grubeck-Loebenstein B. Biology of immune responses to vaccines in elderly persons. Clin Infect Dis. 2008;46:1078–84.
18. Ashwell JD, Lu FW, Vacchio MS. Glucocorticoids in T cell development and function*. Annu Rev Immunol. 2000;18: 309–45.
19. Hanania NA, Sockrider M, Castro M, Holbrook JT, Tonascia J, Wise R, et al. Immune response to influenza vaccination in children and adults with asthma: effect of corticosteroid therapy. J Allergy Clin Immunol. 2004;113:717–24.
20. Bae EY, Choi UY, Kwon HJ, Jeong DC, Rhim JW, Ma SH, et al. Immunogenicity and safety of an inactivated trivalent split influenza virus vaccine in young children with recurrent wheezing. Clin Vaccine Immunol. 2013;20:811–7.
21. Busse WW, Peters SP, Fenton MJ, Mitchell H, Bleecker ER, Castro M, et al. Vaccination of patients with mild and severe asthma with a 2009 pandemic H1N1 influenza virus vaccine. J Allergy Clin Immunol. 2011;127:130–7, 137. e1-3.
22. Mori M, Oura A, Ohnishi H, Washio M. Confounding in evaluating the effectiveness of influenza vaccine. Vaccine. 2008;26:6459–61.
23. Ozasa K. The effect of misclassification on evaluating the effectiveness of influenza vaccines. Vaccine. 2008;26:6462–5.
24. Chartrand C, Leeflang MM, Minion J, Brewer T, Pai M. Accuracy of rapid influenza diagnostic tests: a meta-analysis. Ann Intern Med. 2012;156:500–11.
25. Plotkin SA, Orenstein WA, Offit OA. Vaccines. 6th ed. Elsevier; 2012.
26. Suzuki M, Yoshimine H, Harada Y, Tsuchiya N, Shimada I, Ariyoshi K, et al. Estimating the influenza vaccine effectiveness against medically attended influenza in clinical settings: a hospital-based case-control study with a rapid diagnostic test in Japan. PLoS One. 2013;8:e52103.
27. Kissling E, Valenciano M, Cohen JM, Oroszi B, Barret AS, Rizzo C, et al. I-MOVE multi-centre case control study 2010–11: overall and stratified estimates of influenza vaccine effectiveness in Europe. PLoS One. 2011;6:e27622.

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RESEARCH PAPER

Immunogenicity of trivalent influenza vaccine in patients with lung cancer undergoing anticancer chemotherapy

Kei Nakashima[a], Masahiro Aoshima[a], Satoko Ohfuji[b], Kanzo Suzuki[c], Masahiro Katsurada[a], Naoko Katsurada[a], Masafumi Misawa[a], Yoshihito Otsuka[d], Kyoko Kondo[b], and Yoshio Hirota[e]

[a]Department of Pulmonary Medicine, Kameda Medical Center, Chiba, Japan; [b]Department of Public Health, Osaka City University Graduate School of Medicine, Osaka, Japan; [c]Nagoya City University, School of Nursing, Nagoya, Japan; [d]Department of Laboratory Medicine, Kameda Medical Center, Chiba, Japan; [e]College of Healthcare Management, Fukuoka, Japan

ABSTRACT

Lung cancer is a leading cause of cancer-related death, and patients with lung cancer are a priority group for influenza vaccination. However, few studies have assessed the immunogenicity of the influenza vaccine in these patients. Here, we performed a prospective study to evaluate the immunogenicity of the influenza vaccine in patients with lung cancer undergoing anticancer chemotherapy. Twenty-five patients with lung cancer undergoing anticancer chemotherapy and 26 patients with chronic obstructive pulmonary disease (COPD) as controls were enrolled. A trivalent influenza vaccine containing inactivated A/California/7/2009 (H1N1) pdm09, A/Texas/50/2012 (H3N2), and B/Massachusetts/2/2012 was administered as a single subcutaneous injection. Serum samples were collected before vaccination, and at 4–6 weeks after vaccination. Levels of serum antibody to hemagglutinin were measured. Among patients with lung cancer, the seroprotection rate (postvaccination titer > 1:40) was 84% for both A(H1N1) and A(H3N2), similar to the levels observed in patients with COPD. However, the seroprotection rate for the B strain was significantly lower in patients with lung cancer than in patients with COPD (64% versus 92%). Even after adjustment for potential confounders, patients with lung cancer had a significantly lower odds ratio for seroprotection against the B strain than patients with COPD. Moreover, in patients with lung cancer, those receiving the platinum doublet treatment tended to exhibit a lower seroprotection rate than those receiving a single agent. Thus, patients with lung cancer undergoing anticancer chemotherapy showed acceptable immune responses to a trivalent influenza vaccine, supporting the recommendation for annual influenza vaccination in these patients.

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Introduction

Patients with cancer undergoing systemic chemotherapy have a significant risk of morbidity and mortality from influenza infection.[1,2] According to a previous report,[1] the mortality rate for influenza infection is approximately 9% among patients with cancer. In addition, influenza infection and influenza-related complications often prevent consecutive courses of chemotherapy and worsen the prognosis of these patients.[1,3,4] Therefore, annual influenza vaccination is recommended in patients with cancer for the prevention of severe influenza or related bacterial infections.[2,5,6] Despite this recommendation, prior studies have reported low influenza vaccination rates among patients receiving chemotherapy for cancer.[7,8] The main reasons for the absence of vaccination include lack of recommendation by the treating physician (72%), fear of side effects (33%), and concerns regarding vaccination efficacy (10%).[7] Moreover, information concerning influenza vaccine efficacy, safety, and optimal timing in patients with cancer is limited.[3] However, the immunogenicity of influenza vaccination in cancer patients has been reported in several studies.[3] The reported seroprotection rates (sPs; postvaccination titer > 1:40) of the inactivated influenza vaccine in patients with solid cancers ranges from 38%–78%.[9-11] The intensity and type of chemotherapy and the timing of vaccination in the course of chemotherapy influence the immunogenicity of influenza vaccination.[3]

Lung cancer is the leading cause of cancer-related death among men and women.[12] Owing to the high incidence of lung cancer worldwide, influenza vaccination for this group of patients is crucial. However, to our knowledge, only one study, performed in 1999, has assessed the immunogenicity of influenza vaccine in patients with lung cancer.[9] In addition, in the last few years, the intensity of anticancer chemotherapy has changed with the development and introduction of new drugs such as platinum agents, third-generation drugs, pemetrexed, and bevacizumab.[13]

Therefore, in this study, we aimed to evaluate the immunogenicity of the influenza vaccine in patients with lung cancer undergoing anticancer chemotherapy compared with that in patients with chronic obstructive disease (COPD) as an immunocompetent control. The study subjects were recruited from the Kameda Medical Center, a tertiary care and teaching hospital, which has 925 inpatient beds, 34 clinical departments, and

CONTACT Kei Nakashima kei.7.nakashima@gmail.com Department of Pulmonary Medicine, Kameda Medical Center, 929 Higashi-cho, Kamogawa, Chiba, 296-8602, Japan.

3000 medical staffs. The Kameda Medical Center covers the southern area of Chiba, Japan, which has a population of ~1,000,000. Since immunoresponses are influenced by prevaccination titers, which are related to previous virus exposure (through infection or vaccination), we used logistic regression to analyze models that include potential confounders, including prevaccination titer, as explanatory variables.

Results

A total of 25 patients with lung cancer and 27 patients with COPD were eligible for this study. All patients received one dose of the trivalent influenza vaccine between November and December 2013. Serum samples at 4–6 weeks after vaccination were collected from 25 patients with lung cancer and 26 patients with COPD. One patient with COPD showed a mild rash within 48 h after vaccination, and no other severe adverse events occurred in any of the patients with lung cancer or COPD. During the study period, no subjects reported laboratory-confirmed influenza or influenza-like illness (defined by acute febrile illness [temperature > 38.0°C] with one or more respiratory symptoms [nasal discharge, sore throat, or cough]).

The characteristics of the 51 patients included in the immunogenicity analyses are shown in Table 1. The mean age was 69.4 years, and 41 patients (80.4%) were men. The proportions of patients with pretiter levels of 1:10 or more ranged from 48%–68% in patients with lung cancer and from 69%–88% in patients with COPD. For the A/California/7/2009 (H1N1) pdm09 strain, the percentage of patients with pretiter levels of 1:10 or more was significantly higher in patients with COPD than that in patients with lung cancer. Adenocarcinoma was the most common histological type in patients with lung cancer (40%). Fifteen (60%) patients received chemotherapy with platinum doublet treatment, and 10 (40%) patients received chemotherapy with a single agent.

Table 2 shows the immune responses to the trivalent influenza vaccination among patients with lung cancer or COPD. Patients with lung cancer showed significantly lower geometric mean titers (GMTs) for A/California/7/2009 (H1N1) pdm09 before vaccination than patients with COPD. In the other 2 strains, patients with lung cancer tended to show lower GMTs before vaccination than patients with COPD did. In all strains, GMTs reached the protection levels (≥1:40), and the mean fold rise (MFR) values were over 2.0 fold. The sPs and seroresponse rates (sRs) in patients with lung cancer were 64%–84% and 60%–72%, respectively. In the A/California/7/2009 (H1N1) pdm09 and A/Texas/50/2012 (H3N2) strains, there were no significant differences in sPs between patients with lung cancer and those with COPD. However, the sP of B/Massachusetts/02/2012 in patients with lung cancer was significantly lower than that in patients with COPD (64% vs. 92%, respectively; $p = 0.019$). In all strains, sRs were not significantly different between patients with lung cancer and patients with COPD.

Table 3 shows the odds ratios (ORs) for sRs after trivalent influenza vaccination in patients with lung cancer compared with those in patients with COPD. In all strains, there were no significant reductions in ORs in patients with lung cancer compared with that in patients with COPD, in both the crude analysis and multivariate analysis.

Table 4 shows ORs for seroconversion reats (sCs) after trivalent influenza vaccination in patients with lung cancer compared with those in patients with COPD. For all strains, based on ORs, there were no significant differences between patients with lung cancer and patients with COPD in the multivariate analysis.

Table 5 shows ORs for sPs after trivalent influenza vaccination in patients with lung cancer compared with those in patients with COPD. In B/Massachusetts/02/2012, patients with lung cancer had a significantly lower OR for sP than patients with COPD ($p = 0.028$) in the multivariate analysis. For the other 2 strains, the ORs for sPs were not significantly different between the 2 patient groups.

Table 6 shows the immune responses to trivalent influenza vaccination among patients with lung cancer undergoing anticancer chemotherapy. For A/Texas/50/2012(H3N2), the MFR was 3.6 in patients receiving platinum doublet treatment; this was significantly lower than that in patients receiving treatment with a single agent (11.3; $p = 0.049$). The sPs were lower in patients receiving platinum doublet treatment, although these differences were not significant, for A/California/7/2009 (H1N1) pdm09 (platinum doublet treatment versus single agent treatment: 73% vs. 100%; $p = 0.125$), A/Texas/50/2012 (H3N2) (platinum doublet treatment versus single agent treatment: 73% vs. 100%; $p = 0.125$), and B/Massachusetts/02/2012 (platinum doublet treatment versus single agent treatment: 60% vs. 70%; $p = 0.691$).

Discussion

In this study, we showed that influenza vaccination induced sufficient immune responses in both patients with COPD and those with lung cancer. The immunity after vaccination satisfied the international licensing criteria of the European Agency for the Evaluation of Medical Products and the US Food and Drug Administration.[14,15] For the A/California/7/2009 (H1N1) pdm09 and A/Texas/50/2012 (H3N2) strains, there were no significant differences in the ORs for sP, sR, or sC between patients with COPD and those with lung cancer in the multivariate analysis. However, the OR for the sP of B/Massachusetts/02/2012 was significantly reduced in patients with lung cancer in the multivariate analysis. In addition, the sP, sR, and sC tended to be lower in patients receiving platinum doublet treatment than in patients receiving single agent treatment. Thus, platinum doublet chemotherapy is considered a high-intensity treatment and may suppress the ability of the immune system to make anti-influenza antibodies.

Oncology patients are heterogeneous, and the immunogenicity of the influenza vaccine in patients with cancer differs depending on the type of cancer and the intensity of chemotherapy.[3,16] Some studies have shown that immunization with the influenza vaccine has no benefit in providing adequate seroconversion, particularly in patients with hematological diseases such as lymphomas and multiple myeloma.[17-20] Nevertheless, other studies examining the immunogenicity of vaccination in hematological disease have reported adequate immunoresponses.[21,22] In addition, positive data have been obtained in the majority of studies assessing the immunogenicity of the influenza vaccine in

Table 1. Characteristics of patients with lung cancer or COPD.

Variables		Lung cancer (N = 25)	COPD (N = 26)	*P* value*
Age, years (mean ± SD)		68.0 ± 6.3	70.7 ± 7.4	0.157
Male		18 (72%)	23 (88%)	0.173
Prevaccination titer				
A/California/7/2009(H1N1)pdm09				
	<1:10	13 (52%)	6 (23%)	0.045
	≥1:10	12 (48%)	20 (77%)	
A/Texas/50/2012(H3N2)				
	<1:10	8 (32%)	3 (12%)	0.098
	≥1:10	17 (68%)	23 (88%)	
B/Massachusetts/02/2012				
	<1:10	11 (44%)	8 (30%)	0.393
	≥1:10	14 (56%)	18 (69%)	
Stage of COPD				
GOLD stage 1			7 (27%)	
GOLD stage 2			11 (42%)	
GOLD stage 3			6 (23%)	
GOLD stage 4			2 (8%)	
Histological type of lung cancers				
Adenocarcinoma		10 (40%)		
Squamous cell carcinoma		6 (24%)		
Small cell carcinoma		8 (32%)		
Others		1 (4%)		
Tumor stage				
Stage 1		1 (4%)		
Stage 3		4 (16%)		
Stage 4		17 (68%)		
Recurrence after surgery		2 (8%)		
Recurrence after stereotactic radiotherapy		1 (4%)		
Recent chemotherapy duration of subjects on influenza vaccination (months)				
0–2		10 (40%)		
3–5		6 (24%)		
6–8		1 (4%)		
9–11		2 (8%)		
≥12		6 (24%)		
Chemotherapy in patients with lung cancer		25 (100%)		
Platinum doublet		15 (60%)		
	CDDP + DOC	1 (4%)		
	CDDP + VP-16	2 (8%)		
	CBDCA + PTX	3 (12%)		
	CBDCA + PEM	3 (12%)		
	CBDCA + nab-PTX	2 (8%)		
	CBDCA + TS1	1 (4%)		
	CBDCA + VP-16	3 (12%)		
Single agent		10 (40%)		
	DOC	3 (12%)		
	AMR	3 (12%)		
	PEM	1 (4%)		
	GEM	1 (4%)		
	VNR	1 (4%)		
	PTX	1 (4%)		

Data are expressed as number (%) of patients, unless otherwise indicated.
COPD: chronic obstructive pulmonary disease.
GOLD: Global Initiative for Chronic Obstructive Lung Disease.
CDDP: cisplatin; DOX: docetaxel; VP-16: etoposide; PTX: paclitaxel; PEM: pemetrexed; Nab-PTX: nab-paclitaxel; TS1: tegafur, gimeracil, oteracil potassium; AMR: amrubicin; GEM: gemcitabine; VNR: vinorelbine.
SD: standard deviation.
*P values were calculated by Fisher's exact or student's t tests.

patients with solid tumors such as breast cancer, colorectal cancer, and lung cancer.[9,10,23,24] Although the observed immunogenicity in patients with solid tumors undergoing chemotherapy has been shown to reach protective levels,[9-11,23-25] some reports have shown that the immunogenicity induced by vaccination is lower than that in immunocompetent controls.[10,23] A previous study assessing the immunogenicity of the influenza vaccine in patients with lung cancer showed an sP of 78% (postvaccination titer > 1:40), similar to the seroprotection level of 64%–84% in our study.[9]

In the present study, the sP of B/Massachusetts/02/2012 in patients with lung cancer was 64%, which was significantly lower than that for patients with COPD and was relatively low in comparison with that of the other 2 strains in patients with lung cancer. A previous report showed increased sensitivity and reduced specificity of hemagglutination inhibition tests with an ether-treated influenza B strain.[26] In our study, none of

Table 2. Immune responses to the trivalent influenza vaccine in patients with lung cancer or COPD.

	Geometric mean titer[a]		Mean fold rise[a]	after vaccination[b]		
	Before vaccination (S0)	after vaccination (S1)	S1/S0	Seroprotection rate (sP) (≥1:40): n (%)	Seroresponse rate (sR) (≥4 fold-rise): n (%)	Seroconversion rate (sC) n (%)
A/California/7/2009 (H1N1)pdm09						
Lung cancer	11	105	9.4	21 (84)	18 (72)	18 (72)
COPD	25	123	5.0	21 (81)	13 (50)	11 (42)
	($P = 0.011$)	($P = 0.923$)	($P = 0.132$)	($P = 1.000$)	($P = 0.153$)	($P = 0.048$)
A/Texas/50/2012 (H3N2)						
Lung cancer	19	112	5.7	21 (84)	16 (64)	14 (56)
COPD	37	173	4.7	25 (96)	13 (50)	13 (50)
	($P = 0.079$)	($P = 0.467$)	($P = 0.445$)	($P = 0.191$)	($P = 0.400$)	($P = 0.781$)
B/Massachusetts/02/ 2012						
Lung cancer	13	54	4.2	16 (64)	15 (60)	12 (48)
COPD	22	91	4.2	24 (92)	13 (50)	12 (46)
	($P = 0.141$)	($P = 0.271$)	($P = 0.931$)	($P = 0.019$)	($P = 0.577$)	($P = 1.000$)

[a]Wilcoxon rank-sum test was performed for intercategory comparisons.
[b]Seroprotection rates, seroresponse rates, and seroconversion rates were compared between groups by Fisher's exact test.

the 3 strains was treated with ether. Hence, we believe that the differences in sPs among strains were not derived from variations in methodology. Concerning this relatively poor immune response to B/Massachusetts/02/2012 in patients with lung cancer, our results were similar to those of a prior study, in which the immunogenicity of an inactive quadrivalent influenza vaccine containing the B/Massachusetts/02/2012 strain was evaluated.[27] It is possible that pre-existing immunity to B strains other than B/Massachusetts/02/2012 negatively affected the immune response to the B/Massachusetts/02/2012 strain; this phenomenon is called original antigenic sin.[28,29] For example, a previous study assessing the prime-boost responses following a change in the 2 B lineages (B/Yamagata and B/Victoria) reported the influence of original antigenic sin on responses to B strains; repeated administration of the annual trivalent influenza vaccine containing the B/Victoria lineage antigen strongly recalled antibodies to the B/Yamagata antigen after the first exposure, but elicited lower B/Victoria responses.[30] In Japan, a mixed epidemic of B/Victoria and B/Yamagata was observed during the 2011/2012 and 2012/2013 seasons.[31] During these seasons, some participants in our study could have been sensitized to the B/Victoria or B/Yamagata strain, thereby decreasing immunogenicity to B/Massachusetts/02/2012 (B/Yamagata lineage) owing to the influence of original antigenic sin on responses to B strains. Moreover, the lower prevaccination antibody titer in patients with lung cancer may explain the decreased sP. In the present study, most of the patients with lung cancer had received repetitive chemotherapy at the time of vaccination, potentially strengthening the lower GMTs before vaccination owing to the immunosuppression caused by chemotherapy.

In this study, we chose patients with COPD as the immunocompetent control group because they are also a priority group for influenza vaccination,[32,33] and are considered immunocompetent compared to patients with cancer undergoing chemotherapy. However, whether patients with COPD are suitable as a control group may be controversial because there is insufficient high-quality evidence on current influenza vaccine regimens for patients with COPD.[34] In a prior study, patients with COPD and healthy controls obtained seroprotective levels of antibodies after influenza vaccination, although the sR seemed to be lower in patients with COPD than in healthy participants, because of the higher prevaccination titer in patients with COPD.[35] In addition, our study showed that the sPs among patients with COPD reached 81%–96% after vaccination. Thus, the patients with COPD in our study appeared to be appropriate as a control group.

Our study has several limitations. First, we had a relatively small sample size. Enrollment of patients was restricted by the

Table 3. Odds ratios for seroresponse rates at after trivalent influenza vaccination in patients with lung cancer or COPD.

	Seroresponse rate (sR) (≥4 fold-rise): n (%)	Crude analysis		Multivariate analysis*	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
A/California/7/2009(H1N1)pdm09					
Lung cancer	18 (72)	2.52 (0.70–9.79)	0.153	1.29 (0.27–6.29)	0.749
COPD	13 (50)	1.00 (ref.)		1.00 (ref.)	
A/Texas/50/2012(H3N2)					
Lung cancer	16 (64)	1.76 (0.51–6.35)	0.400	1.11 (0.30–4.15)	0.872
COPD	13 (50)	1.00 (ref.)		1.00 (ref.)	
B/Massachusetts/02/2012					
Lung cancer	15 (60)	1.49 (0.43–5.25)	0.577	1.02 (0.28–3.78)	0.975
COPD	13 (50)	1.00 (ref.)		1.00 (ref.)	

*Adjusted for age, gender, and prevaccination titer.

Table 4. Odds ratios for seroconversion rates after trivalent influenza vaccination in patients with lung cancer or COPD.

	Seroconversion rate (sC): n (%)	Crude analysis		Multivariate analysis*	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
A/California/7/2009(H1N1)pdm09					
Lung cancer	18 (72)	3.42 (0.95–13.4)	0.048	2.48 (0.62–9.99)	0.200
COPD	11 (42)	1.00 (ref.)		1.00 (ref.)	
A/Texas/50/2012(H3N2)					
Lung cancer	14 (56)	1.27 (0.37–4.41)	0.781	0.88 (0.25–3.1)	0.847
COPD	13 (50)	1.00 (ref.)		1.00 (ref.)	
B/Massachusetts/02/2012					
Lung cancer	12 (48)	1.08 (0.31–3.71)	1.000	0.76 (0.22–2.6)	0.663
COPD	12 (46)	1.00 (ref.)		1.00 (ref.)	

*Adjusted for age, gender, and prevaccination titer.

short period from the time when the influenza vaccine became available to vaccination before the anticipated winter outbreak of influenza infection. Inclusion of more cases may provide more definitive results, particularly when considering the effects of different chemotherapy regimens on patients with lung cancer. Second, in our study, there was the possibility of intercurrent asymptomatic infections. However, we monitored all patients for influenza-like illness, and no patients experienced confirmed influenza virus infections during the study period. Thus, we believe that the effect of intercurrent infection was not large enough to invalidate the present results. Third, healthy controls were not evaluated in this study. However, we evaluated patients with COPD as a immunocompetent control group; these patients were considered appropriate based on their high sPs. Fourth, the antibody titers at the end of season were not evaluated in the present study because we wanted to focus on the antibody titers before vaccination and at 4–6 weeks after vaccination. We thought that the immunogenicity at 4–6 weeks after vaccination was clinically important for evaluating the immunoprotective ability against influenza infection during the influenza season.

In conclusion, patients with lung cancer undergoing chemotherapy showed an acceptable immune response to the trivalent influenza vaccine without significant adverse effects, supporting the recommendation for annual influenza vaccination in patients with lung cancer. Further studies assessing influenza vaccine efficacy in patients with cancer are needed to strengthen the evidence supporting influenza vaccination in these patients.

Materials and methods

Study design and patients

In November and December 2013, patients with lung cancer undergoing chemotherapy and patients with COPD being treated at the Department of Pulmonary Medicine, Kameda Medical Center, Chiba, Japan were invited to participate in this study. We chose patients with COPD as immunocompetent controls because these patients are a priority group for influenza vaccination,[32,33] and most patients with COPD receive the influenza vaccine annually in Japan.

The inclusion criteria were as follows: 1) patients with lung cancer who underwent anticancer chemotherapy with cytotoxic agents from 2 weeks before influenza vaccination to 4 weeks after vaccination, or 2) patients with COPD diagnosed based on a postbronchodilator forced expiratory volume in 1 second/forced vital capacity (FEV_1 /FVC) of less than 70%.[36] Exclusion criteria were as follows: a history of influenza infection, an acute febrile illness or evidences of severe acute illness at the time of vaccination, a history of allergy due to vaccine components, or other contraindications for receiving the vaccine.

All patients provided written informed consent after the nature and possible consequences of the study were explained. The study protocol was approved by the Research Ethics Committee of the Kameda Medical Center (No. 13–061) and was performed in accordance with the Declaration of Helsinki. After obtaining informed consent, the baseline patient characteristics, including age, gender, histological type of lung cancer, clinical stage of lung cancer, stage of COPD according to the

Table 5. Odds ratios for seroprotection rates at after trivalent influenza vaccination in patients with lung cancer or COPD.

	Seroprotection rate (sP) (≥1:40): n (%)	Crude analysis		Multivariate analysis*	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
A/California/7/2009(H1N1)pdm09					
Lung cancer	21 (84)	1.24 (0.23–7.21)	1.000	1.56 (0.28–8.84)	0.613
COPD	21 (81)	1.00 (ref.)		1.00 (ref.)	
A/Texas/50/2012(H3N2)					
Lung cancer	21 (84)	0.22 (0.00–2.40)	0.191	0.30 (0.02–4.01)	0.364
COPD	25 (96)	1.00 (ref.)		1.00 (ref.)	
B/Massachusetts/02/2012					
Lung cancer	16 (64)	0.15 (0.01–0.88)	0.019	0.11 (0.01–0.79)	0.028
COPD	24 (92)	1.00 (ref.)		1.00 (ref.)	

*Adjusted for age, gender, and prevaccination titer.

Table 6. Immune response to the trivalent influenza vaccine in patients with lung cancer, according to the type of chemotherapy.

	Geometric mean titer[a]		Mean fold rise[a]	After vaccination[b]		
	Before vaccination (S0)	After vaccination (S1)	S1/S0	Seroprotection rate (sP) (≥1:40): n (%)	Seroresponse rate (sR) (≥4 fold-rise): n (%)	Seroconversion rate (sC) : n (%)
A/California/7/2009(H1N1) pdm09						
Single agent	12	160	13.0	10 (100)	8 (80)	8 (80)
Platinum doublet	10	80	7.6	11 (73)	10 (67)	10 (67)
	($P = 0.809$)	($P = 0.306$)	($P = 0.415$)	($P = 0.125$)	($P = 0.659$)	($P = 0.659$)
A/Texas/50/2012(H3N2)						
Single agent	16	184	11.3	10 (100)	8 (80)	8 (80)
Platinum doublet	22	80	3.6	11 (73)	8 (53)	6 (40)
	($P = 0.590$)	($P = 0.143$)	($P = 0.049$)	($P = 0.125$)	($P = 0.229$)	($P = 0.099$)
B/Massachusetts/02/2012						
Single agent	12	80	6.5	7 (70)	8 (80)	5 (50)
Platinum doublet	13	42	3.2	9 (60)	7 (47)	7 (47)
	($P = 0.254$)	($P = 0.324$)	($P = 0.122$)	($P = 0.691$)	($P = 0.211$)	($P = 1.000$)

[a]Wilcoxon rank-sum tests were performed for intercategory comparisons.
[b]Seroprotection rates, seroresponse rates, and seroconversion rates were compared between groups by Fisher's exact test.

Global Initiative for Chronic Obstructive Lung Disease criteria,[33,36] smoking status, and type of chemotherapy, were collected.

Vaccination

FLUBIK HA Syringe (Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan) containing inactivated A/California/7/2009 (H1N1) pdm09, A/Texas/50/2012 (H3N2), and B/ Massachusetts/2/2012 (B/Yamagata lineage) was administered as a single subcutaneous injection, the typical administration route used in Japan. Each vaccine contained 15 μg hemagglutinin antigen of each strain. The vaccine did not contain thimerosal. The vaccine was prepared in embryonated chicken eggs using standard methods for the production of trivalent inactivated vaccine. Attending physicians monitored all patients until April 2014 and evaluated the occurrence of side effects from the vaccine or of influenza infection.

Measurement of antibody titers

Serum samples were collected before vaccination (S0) and at 4–6 weeks after vaccination (S1). All serum specimens were kept at −40°C until analysis. Serum antibody levels to hemagglutinin were measured according to the standard microtiter hemagglutination inhibition method[37] with the same antigens as used in the vaccine. During the measurement process, none of the 3 antigens was treated with ether. All samples were assayed at the Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan in June 2014.

Statistical analyses

The following outcomes were calculated to evaluate the immunogenicity of influenza vaccine: GMT, MFR, sP (postvaccination titer > 1:40), and sR (> 4-fold rise), sC (a prevaccination titer < 1:10 and a postvaccination titer ≥ 1:40 or a prevaccination titer ≥ 1:10 and a minimum 4-fold rise in postvaccination titer). During data processing, titers of less than 1:10 were regarded as 1:5, and reciprocal antibody titers were analyzed after logarithmic transformation. The results are presented in the original scale by calculating the antilogarithm. Stratified analyses were performed to examine the effects of the following potential confounders: age at vaccination (<69 and ≥69 years), gender (men versus women), prevaccination titer (<1:10 and ≥1:10), and underlying diseases (lung cancer and COPD). The significance of fold rise within a category was assessed by the Wilcoxon signed-rank test, and intercategory comparisons were made by the Wilcoxon rank-sum test. Student's t tests or Fisher's exact tests were performed where appropriate. In addition, the independent effects of potential confounders on antibody induction were assessed by logistic regression. The models were constructed with sP, sR, and sC as the dependent variables and with the above-mentioned potential confounders as the explanatory variables. ORs and 95% confidence intervals (CIs) were calculated. All tests were 2-sided, and all analyses were performed using R version 3.2.3 (R foundation for Statistical Computing; http://www.r-project.org). Differences with p values less than 0.05 were considered significant.

Abbreviations

COPD	chronic obstructive pulmonary disease
sP	seroprotection rate
GMT	geometric mean titer
MFR	mean fold rise
sR	seroresponse rate
OR	odds ratio
95% CI	95% confidence interval

Disclosure of potential conflicts of interest

The authors declare that they have no conflicts of interest.

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References

[1] Cooksley CD, Avritscher EB, Bekele BN, Rolston KV, Geraci JM, Elting LS. Epidemiology and outcomes of serious influenza-related infections in the cancer population. Cancer 2005; 104:618-28; PMID:15973737; http://dx.doi.org/10.1002/cncr.21203

[2] Kunisaki KM, Janoff EN. Influenza in immunosuppressed populations: a review of infection frequency, morbidity, mortality, and vaccine responses. Lancet Infect Dis 2009; 9:493-504; PMID:19628174; http://dx.doi.org/10.1016/S1473-3099(09)70175-6

[3] Shehata MA, Karim NA. Influenza vaccination in cancer patients undergoing systemic therapy. Clin Med Insights Oncol 2014; 8:57-64; PMID:24855405

[4] Earle CC. Influenza vaccination in elderly patients with advanced colorectal cancer. J Clin Oncol 2003; 21:1161-6; PMID:12637485; http://dx.doi.org/10.1200/JCO.2003.06.008

[5] Beck CR, McKenzie BC, Hashim AB, Harris RC, Zanuzdana A, Agboado G, Orton E, Bechard-Evans L, Morgan G, Stevenson C, et al. Influenza vaccination for immunocompromised patients: systematic review and meta-analysis from a public health policy perspective. PLoS One 2011; 6:e29249; PMID:22216224; http://dx.doi.org/10.1371/journal.pone.0029249

[6] Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, Bousvaros A, Dhanireddy S, Sung L, Keyserling H, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis 2014; 58:309-18; PMID:24421306; http://dx.doi.org/10.1093/cid/cit816

[7] Loulergue P, Mir O, Alexandre J, Ropert S, Goldwasser F, Launay O. Low influenza vaccination rate among patients receiving chemotherapy for cancer. Ann Oncol 2008; 19:1658; PMID:18662953; http://dx.doi.org/10.1093/annonc/mdn531

[8] Meidani M, Rostami M, Dehghani F. Why coverage of influenza vaccine is not enough in patients receiving chemotherapy? Int J Prev Med 2011; 2:186-7; PMID:21811662

[9] Anderson H, Petrie K, Berrisford C, Charlett A, Thatcher N, Zambon M. Seroconversion after influenza vaccination in patients with lung cancer. Br J Cancer 1999; 80:219-20; PMID:10389999; http://dx.doi.org/10.1038/sj.bjc.6690342

[10] Kim DH, Lee YY, Shin US, Moon SM. Immunogenicity of influenza vaccine in colorectal cancer patients. Cancer Res Treat 2013; 45:303-12; PMID:24454003; http://dx.doi.org/10.4143/crt.2013.45.4.303

[11] Xu Y, Methuku N, Coimbatore P, Fitzgerald T, Huang Y, Xiao YY, Pagala M, Gupta S, Solomon W, Rubin P, et al. Immunogenicity of an inactivated monovalent 2009 influenza A (H1N1) vaccine in patients who have cancer. Oncologist 2012; 17:125-34; PMID:22240540; http://dx.doi.org/10.1634/theoncologist.2011-0220

[12] Global Burden of Disease Cancer Collaboration. Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, Allen C, Hansen G, Woodbrook R, et al. The Global Burden of Cancer 2013. JAMA Oncol 2015; 1:505-27; PMID:26181261; http://dx.doi.org/10.1001/jamaoncol.2015.0735

[13] Masters GA, Temin S, Azzoli CG, Giaccone G, Baker S, Jr, Brahmer JR, Ellis PM, Gajra A, Rackear N, Schiller JH, et al. Systemic Therapy for Stage IV Non-Small-Cell Lung Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. J Clin Oncol 2015; 33:3488-515; PMID:26324367; http://dx.doi.org/10.1200/JCO.2015.62.1342

[14] European Committee for Proprietary Medial Products. Note for Guidance on Harmonisation of Requirements for Influenza Vaccines (CPMP/BWP/214/96). London: European Agency Evaluation Medical Products, 1997.

[15] Center for Biologics Evaluation and Research. Guidance for industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines. Food Drug Administration, 2007.

[16] Beck CR, McKenzie BC, Hashim AB, Harris RC, University of Nottingham I, the ImmunoCompromised Study G, Nguyen-Van-Tam JS. Influenza vaccination for immunocompromised patients: systematic review and meta-analysis by etiology. J Infect Dis 2012; 206:1250-9; PMID:22904335; http://dx.doi.org/10.1093/infdis/jis487

[17] Robertson JD, Nagesh K, Jowitt SN, Dougal M, Anderson H, Mutton K, Zambon M, Scarffe JH. Immunogenicity of vaccination against influenza, Streptococcus pneumoniae and Haemophilus influenzae type B in patients with multiple myeloma. Br J Cancer 2000; 82:1261-5; PMID:10755398; http://dx.doi.org/10.1054/bjoc.1999.1088

[18] Ljungman P, Nahi H, Linde A. Vaccination of patients with haematological malignancies with one or two doses of influenza vaccine: a randomised study. Br J Haematol 2005; 130:96-8; PMID:15982350; http://dx.doi.org/10.1111/j.1365-2141.2005.05582.x

[19] Mazza JJ, Yale SH, Arrowood JR, Reynolds CE, Glurich I, Chyou PH, Linneman JG, Reed KD. Efficacy of the influenza vaccine in patients with malignant lymphoma. Clin Med Res 2005; 3:214-20; PMID:16303886; http://dx.doi.org/10.3121/cmr.3.4.214

[20] Yri OE, Torfoss D, Hungnes O, Tierens A, Waalen K, Nordoy T, Dudman S, Kilander A, Wader KF, Ostenstad B, et al. Rituximab blocks protective serologic response to influenza A (H1N1) 2009 vaccination in lymphoma patients during or within 6 months after treatment. Blood 2011; 118:6769-71; PMID:22058114; http://dx.doi.org/10.1182/blood-2011-08-372649

[21] Hodges GR, Davis JW, Lewis HD, Jr, Whittier FC, Jr, Siegel CD, Chin TD, Clark GM, Noble GR. Response to influenza A vaccine among high-risk patients. South Med J 1979; 72:29-32; PMID:366766; http://dx.doi.org/10.1097/00007611-197901000-00010

[22] Brydak LB, Machala M, Centkowski P, Warzocha K, Bilinski P. Humoral response to hemagglutinin components of influenza vaccine in patients with non-Hodgkin malignant lymphoma. Vaccine 2006; 24:6620-3; PMID:16870313; http://dx.doi.org/10.1016/j.vaccine.2006.05.100

[23] Puthillath A, Trump DL, Andrews C, Bir A, Romano K, Wisniewski M, Fakih MG. Serological immune responses to influenza vaccine in patients with colorectal cancer. Cancer Chemother Pharmacol 2011; 67:111-5; PMID:20204362; http://dx.doi.org/10.1007/s00280-010-1292-2

[24] Brydak LB, Guzy J, Starzyk J, Machala M, Gozdz SS. Humoral immune response after vaccination against influenza in patients with breast cancer. Support Care Cancer 2001; 9:65-8; PMID:11147146; http://dx.doi.org/10.1007/s005200000186

[25] Vilar-Compte D, Cornejo P, Valle-Salinas A, Roldan-Marin R, Iguala M, Cervantes Y, Lopez I. Influenza vaccination in patients with breast cancer: a case-series analysis. Med Sci Monit 2006; 12:CR332-6; PMID:16865064

[26] Kendal AP, Cate TR. Increased sensitivity and reduced specificity of hemagglutination inhibition tests with ether-treated influenza B/Singapore/222/79. J Clin Microbiol 1983; 18:930-4; PMID:6630472

[27] Tsurudome Y, Kimachi K, Okada Y, Matsuura K, Ooyama Y, Ibaragi K, Kino Y, Ueda K. Immunogenicity and safety of an inactivated quadrivalent influenza vaccine in healthy adults: a phase II, open-label, uncontrolled trial in Japan. Microbiol Immunol 2015; 59:597-604. accessed at ; PMID:26272602; http://dx.doi.org/10.1111/1348-0421.12316

[28] Francis T, Salk JE, Quilligan JJ. Experience with vaccination against influenza in the spring of 1947: A preliminary report. Am J Public Health Nations Health 1947; 37:1013-6; http://dx.doi.org/10.2105/AJPH.37.8.1013

[29] Ndifon W. A simple mechanistic explanation for original antigenic sin and its alleviation by adjuvants. J R Soc Interface 2015; 12:20150627; PMID:26577593; http://dx.doi.org/10.1098/rsif.2015.0627

[30] Skowronski DM, Hottes TS, De Serres G, Ward BJ, Janjua NZ, Sabaiduc S, Chan T, Petric M. Influenza B/Victoria antigen induces strong recall of B/Yamagata but lower B/Victoria response in children primed with two doses of B/Yamagata. Pediatr Infect Dis J 2011; 30:833-9; PMID:21857263; http://dx.doi.org/10.1097/INF.0b013e31822db4dc

[31] Ministry of Health, Labour and Welfare, Japan. Stratgey for prevention of influenza infection. Available at: http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou01/dl/fludoco1415.pdf (accessed June 25, 2016)
[32] Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, Mootrey G, Bresee JS, Cox NJ, Centers for Disease C, Prevention. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. MMWR Recommendations Reports 2009; 58:1-52.
[33] Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015. Available from: http://www.goldcopd.org/
[34] Poole PJ, Chacko E, Wood-Baker RW, Cates CJ. Influenza vaccine for patients with chronic obstructive pulmonary disease. Cochrane Database Syst Rev 2006; CD002733; PMID:16437444
[35] Nath KD, Burel JG, Shankar V, Pritchard AL, Towers M, Looke D, Davies JM, Upham JW. Clinical factors associated with the humoral immune response to influenza vaccination in chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2014; 9:51-6; PMID:24399872
[36] Qaseem A, Snow V, Shekelle P, Sherif K, Wilt TJ, Weinberger S, Owens DK, Clinical Efficacy Assessment Subcommittee of the American College of P. Diagnosis and management of stable chronic obstructive pulmonary disease: a clinical practice guideline from the American College of Physicians. Annals Internal Med 2007; 147:633-8; PMID:17975186; http://dx.doi.org/10.7326/0003-4819-147-9-200711060-00008
[37] Hirst GK. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. J Exp Med 1942; 75:49-64; PMID:19871167; http://dx.doi.org/10.1084/jem.75.1.49

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ORIGINAL ARTICLE—ALIMENTARY TRACT

Booster influenza vaccination does not improve immune response in adult inflammatory bowel disease patients treated with immunosuppressives: a randomized controlled trial

Hiroko Matsumoto · Satoko Ohfuji · Kenji Watanabe · Hirokazu Yamagami · Wakaba Fukushima · Kazuhiro Maeda · Noriko Kamata · Mitsue Sogawa · Masatsugu Shiba · Tetsuya Tanigawa · Kazunari Tominaga · Toshio Watanabe · Yasuhiro Fujiwara · Yoshio Hirota · Tetsuo Arakawa

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Abstract
Background This research was conducted is to assess the effect of booster doses of the trivalent influenza vaccine in adult inflammatory bowel disease (IBD) patients treated with anti-tumor necrosis factor (TNF)-α agents and/or immunomodulators.
Methods Adult IBD patients and healthy individuals were subcutaneously administered the trivalent influenza vaccine. They were randomized into two groups: the single vaccination group and the two vaccination booster group. Blood samples were collected, and the antibody titers against each influenza strain were determined by hemagglutination inhibition at 3 different time points (pre-vaccination, 3 weeks post-vaccination, and after the flu season) in the single vaccination group and at 4 time points (pre-vaccination, 3 weeks post-first vaccination, 3 weeks post-second vaccination, and after the flu season) in the booster vaccination group.
Results Seventy-eight IBD patients and 11 healthy controls were randomized into the single vaccination group and the booster vaccination group. Twenty-nine patients received immunomodulators; 21 received anti-TNF-α agents; and 28 received a combination of both. No significant differences were observed in the evaluated immune response parameters between 3 weeks post-vaccination in the single vaccination group and 3 weeks post-second vaccination in the booster vaccination group (geometric mean titers: H1N1, $p = 0.09$; H3N2: $p = 0.99$; B: $p = 0.94$). A higher pre-vaccination titer was significantly associated with sufficient seroprotection rate after vaccination for the H1N1 strain (odds ratio 11.93, $p = 0.03$).
Conclusions The second booster of trivalent influenza vaccination did not improve the immune response in adult IBD patients who were treated with immunomodulators and/or anti-TNF-α agents.

Keywords Inflammatory bowel disease · Immunomodulator · Anti-tumor necrosis factor-α agent · Booster vaccination · Influenza vaccine

Abbreviations

ADA	Adalimumab
AZA	Azathioprines
CD	Crohn's disease
GMT	The geometric mean titer
HAI	Hemagglutination inhibition
HBI	Harvey–Bradshaw index
HBV	Hepatitis B virus
IBD	Inflammatory bowel disease
IFX	Infliximab
OR	Odds ratio
TNF-α	Anti-tumor necrosis factor-α
UC	Ulcerative colitis

H. Matsumoto · K. Watanabe (✉) · H. Yamagami · N. Kamata · M. Sogawa · M. Shiba · T. Tanigawa · K. Tominaga · T. Watanabe · Y. Fujiwara · T. Arakawa
Department of Gastroenterology, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan
e-mail: m1158756@med.osaka-cu.ac.jp

S. Ohfuji · W. Fukushima · Y. Hirota
Department of Public Health, Osaka City University Graduate School of Medicine, Osaka, Japan

K. Maeda
Research Foundation for Microbial Disease of Osaka University, Osaka, Japan

Y. Hirota
Clinical Epidemiology Research Center, Medical Co. LTA, Fukuoka, Japan

UMIN-CTR University Hospital Medical Information Network Clinical Trial Registry
6MP 6-Mercaptopurine

Introduction

Patients with inflammatory bowel diseases (IBDs) such as ulcerative colitis (UC), Crohn's disease (CD), and intestinal Behçet's disease have chronic intestinal inflammation from various causes of environmental factors, dysregulated immune systems, and genetic susceptibility [1]. Immunosuppressive therapy, immunomodulators, or anti-tumor necrosis factor (TNF)-α agents are currently used in IBD patients to improve clinical outcomes with remission induction and maintenance; however, these treatments can increase adverse events, including infections [2–4]. In particular, elderly IBD patients may be at increased risk for opportunistic infections [5, 6].

Influenza is an annual respiratory infection that can cause serious complications. In the United States, influenza causes about 226,000 hospitalizations and about 36,000 related deaths every year [7, 8]. Patients who are compromised, elderly, or treated with immunosuppressive agents are at a higher risk of having complications if they are infected with the influenza virus [9]. Therefore, it is recommended for IBD patients who are treated with immunosuppressive agents to get the annual influenza vaccination [9].

We previously reported that immune responses to the trivalent influenza vaccination were inhibited for some strains in adult IBD patients who were treated with infliximab (IFX) and/or immunomodulators [10]. This has also been reported in pediatric IBD patients [11].

Children generally receive two trivalent influenza vaccinations in one season because of their immunogenicity [12–14]. A second booster influenza vaccination is effective in children for improving immune responses after insufficient immune responses following the first vaccination [15]. However, it has not been clarified whether a first and a second booster influenza vaccination might be less effective for adult IBD patients treated with IFX and/or IM in comparison with healthy controls.

Pediatric IBD patients treated with IFX are highly susceptible to hepatitis B virus (HBV) reactivation; thus, clinicians need to screen for HBV immunity when they are diagnosed with IBD. The HBV vaccine, the same inactive vaccine, showed an anamnestic immune response after the second booster vaccination [16]. In patients with rheumatoid arthritis who are receiving treatment with immunosuppressive drugs, optimization with a booster dose of the trivalent influenza vaccine is also considered [17, 18]. We conducted the first prospective randomized controlled study to evaluate the efficacy of booster doses of the trivalent influenza vaccination in adult IBD patients who were treated with anti-TNF-α agents and/or immunomodulators.

Methods

Subjects

We conducted a prospective, open label, randomized, controlled, parallel-group comparison study from November 2012 to July 2013 in the Department of Gastroenterology at the Osaka City University Hospital. The study protocol was approved by the Ethics Review Board of the Osaka City University Graduate School of Medicine, and it was registered at the University Hospital Medical Information Network Clinical Trial Registry in advance (UMIN000009259).

Study subjects consisted of IBD patients receiving immunosuppressive therapy, immunomodulators and/or anti-TNF-α agents, and healthy volunteers were the controls (≥20 years). The exclusion criteria were as follows: (1) subjects who had already received the 2012 trivalent inactivated influenza vaccine; (2) subjects with a history of influenza infection within the last 6 months; and (3) subjects with a history of anaphylactic reaction to a previous influenza vaccine or vaccine components, or an acute febrile illness or signs of severe acute illness at the time of vaccination. All subjects provided written informed consent after the study design and possible risks were explained. We estimated that the appropriate sample size for the primary objective was 108 IBD patients and 20 controls. This was based on the assumption of a 2.5 odds ratio (OR) for an appropriate immune response in the booster two vaccination group compared to the single vaccination group, according to the data of our preliminary study, and a power of 80 % and an alpha of 0.05. The IBD patients were randomized into a single or booster vaccination group with a 1:1 ratio, allocation for age (<49 and ≥49 years), and the type of immunosuppressive therapy (i.e., immunomodulator monotherapy, anti-TNF-α agent monotherapy, or a combination of both). The controls were randomized into the single vaccination group or booster vaccination group.

Data collection

At the time of recruitment, we collected the following clinical information from the IBD patients' medical records: age, sex, diagnosed disease (UC, CD, or intestinal

Behçet's disease), duration of disease, current therapy [azathioprines (AZA), 6-mercaptopurine (6MP), IFX, and adalimumab (ADA)] that has been continued for >3 -months, disease activity [UC: partial Mayo score; CD: Harvey–Bradshaw index (HBI)]. A partial Mayo score of ≤2 for UC and HBI of ≤4 for CD are defined as remission stage.

Before vaccination, the subjects were asked to complete a self-administered questionnaire, which collected the following information: age at vaccination, body height and weight, underlying illnesses, past medical history, and allergic history (including allergy to eggs).

Vaccination with the trivalent vaccine

Each subject received a single dose or two doses as a booster of the 2012–2013 seasonal trivalent inactivated influenza vaccine (Lot HA119E; Biken, Osaka, Japan) subcutaneously. In Japan, subcutaneous administration is the routine for influenza vaccinations. The vaccine strains were A/California/7/2009 (H1N1) pdm09, A/Victoria/361/2011 (H3N2), and B/Wisconsin/01/2010 (B). A standard 0.5 mL dose of the vaccine contained 15 μg of the hemagglutinin antigen of each strain. For the booster vaccination group, the subjects received a second vaccination after 3 weeks from the first vaccination.

Measurement of hemagglutination inhibition antibody titers

Figure 1 presents an outline of the present study design. Serum samples were collected at 4 time points in the booster vaccination group: before vaccination (S0), 3 weeks after the first dose (S1), 3 weeks after the second dose (S2), and after the influenza season (after April 2013; S3). For the single vaccination group, the serum samples were collected at the following 3 time points: before vaccination (S0), 3 weeks after the first dose (S1), and after the influenza season (after April 2013; S3). All serum specimens were stored at −80 °C until they were tested for hemagglutination inhibition (HAI) antibody titers against all strains simultaneously. The HAI antibodies were measured using the standard microtiter HAI method with the same antigens as in the vaccine [19]. All samples were measured at the laboratory of the Research Foundation for Microbial Disease of Osaka University between July 2013 and September 2013.

Statistical analyses

The following outcomes were calculated to assess the immunogenicity of the influenza vaccine: the geometric mean titer (GMT), mean fold-rise, seroresponse rate (≥4-fold rise), and seroprotection rate (HI titer ≥1:40). For data processing, titers <1:10 were regarded as 1:5, and reciprocal antibody titers were analyzed after logarithmic transformation. The results were presented in the original scale by calculating the antilogarithm. A stratified analysis was also performed to investigate the effect of potential confounders: sex, age at vaccination, disease duration, immunosuppressive treatment, defined disease, disease activity (remission or active), and pre-vaccination titer (<1:10, 1:10–1:20, and ≥1:40). The significance of the fold-rise within a category was assessed using the Wilcoxon signed-rank test, and inter-category comparisons were made by using either the Wilcoxon rank-sum or Kruskal–Wallis tests. The Chi square test or Mantel-extension method for the trend test was also used when appropriate. Furthermore, to consider the independent effect of the booster dose on the immune response, multivariate analyses were conducted using logistic regression models with potential confounders. We chose to adjust the variables,

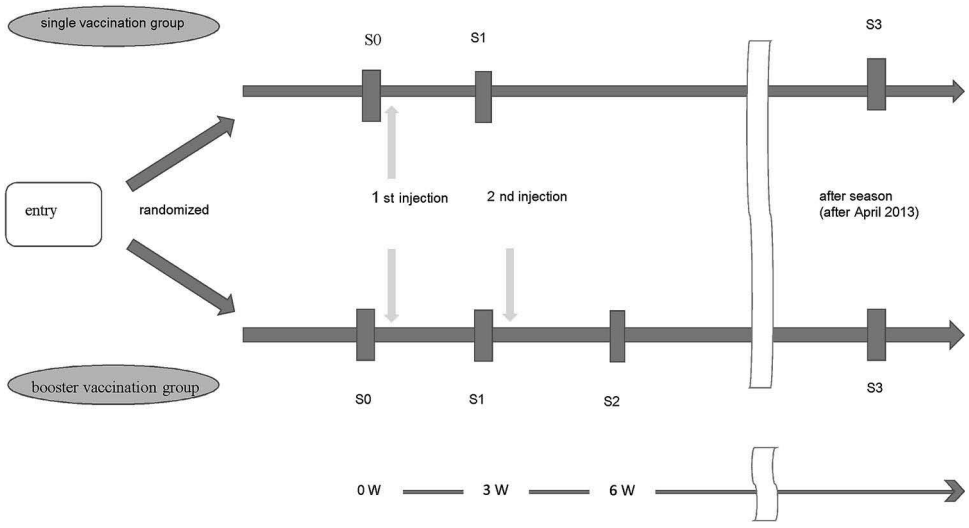

Fig. 1 An outline of the present study design

which revealed the differences in the stratified analysis, or we reported the effect on the immune response from previous studies, as potential confounders. All analyses were performed using SAS, version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Study participants

Seventy-eight IBD patients and 11 controls were enrolled. The baseline characteristics were well matched for sex, age at vaccination, disease, and disease duration between the two groups after randomization (Table 1). The immunosuppressive therapy, immunomodulator monotherapy, anti-TNF-α agent monotherapy, and combination therapy were also randomized well between the two groups ($p = 0.82$). Thirty-eight patients had CD, 33 had UC, and 7 had Behçet's disease. There were no statistically significant differences in disease activity between the two groups (HBI: $p = 0.66$; partial Mayo score: $p = 0.19$).

Forty-six participants received a single dose of the influenza vaccination and 43 participants received two doses between November 5, 2012 and December 28, 2012. All 89 subjects had follow up until July 2013.

Changes in the parameters of immunogenicity

The immune responses to the trivalent influenza vaccination for the 3 strains during each phase are shown in Table 2. Among 78 IBD patients, there were no significant differences in GMTs after vaccination between S1 in the single vaccination group and S2 in the booster vaccination group (H1N1: $p = 0.09$; H3N2: $p = 0.99$; B: $p = 0.94$). There were also no significant differences in GMTs after the flu season for each strain between the groups (H1N1: $p = 0.54$; H3N2: $p = 0.93$; B: $p = 0.90$). Although the seroprotection rate for the H1N1 strain after two vaccinations (S2) was lower in the booster vaccination group than that (S1) in the single vaccination group ($p = 0.04$), the seroprotection rates for the other strains were similarly observed in both groups. The seroprotection rates after the flu season were equally distributed for each strain between the groups (H1N1: $p = 0.35$; H3N2: $p = 0.80$; B: $p = 0.31$).

In the study subjects, the pre-vaccination titer for the B strain was very high compared to that of the H1N1 or H3N2 strains. Therefore, the seroprotection rates of the B strain for all participants after vaccination (S1 or S2) were 100 %.

In the single vaccination group, the seroprotection rate after vaccination (S1) was >70 % for every strain (H1N1: 85 %, H3N2: 82 %, B: 100 %). This means that the trivalent 2012/2013 seasonal influenza vaccine used in this study provided sufficient immune responses from a single vaccination, even though the IBD patients were treated with immunosuppressive agents [14].

Among the control subjects, there were no significant differences between the single vaccination and booster vaccination groups with regard to the GMT, fold-rise, and seroprotection rate at each point. In the control group, a relatively low pre-vaccination titer (≤1:20) was noted for H1N1 in 5 participants, for H3N2 in 9 participants, and for B in 0 participants. However, only one participant was unexpectedly randomized into the booster vaccination group; the GMT of this patient after the second vaccination did not improve.

Stratified immunogenicity analysis

To focus on the influences of the type of immunosuppressive treatment or pre-vaccination titer for the immune responses of the 3 strains at each phase, we performed a stratified analysis of the single vaccination and booster vaccination groups (Table 3). However, there were no associations between the types of immunosuppressive treatment (AZA or 6MP, IFX or ADA, AZA/6MP, and IFX/ADA) or the immune responses between the groups after vaccination (S1 in the single vaccination group vs. S2 in the booster vaccination group). Figure 2a shows the changing process of GMTs for the 3 strains according to the types of immunosuppressive treatment at each phase in only the booster vaccination group. The second booster vaccination did not influence GMTs in relation to the type of immunosuppressive treatment in adult IBD patients.

Conversely, subjects with a higher pre-vaccination titer showed higher GMTs and seroprotection rates for H1N1 and H3N2 strains after vaccination (S1 or S2; Table 3), as described in our previous study [10]. Figure 2b shows the changing process of GMT for the 3 strains according to the pre-vaccination titer in the booster group. All participants had a higher pre-vaccination titer (≥1:40) for the B strain. The second booster vaccination did not improve the GMT of the lower pre-vaccination titer group (<1:40) in adult IBD patients who were treated with immunosuppressive agents. Additionally, Fig. 2c shows the changing process of GMT for the 3 strains according to the immune response at S1 after the first vaccination in the booster vaccination group. The second booster vaccination also did not improve the GMT of the lower immune response group (<1:40) in adult IBD patients who were treated with anti-TNF-α agents and/or immunomodulators.

After adjusting for immunosuppressive therapy and the pre-vaccination titer, the booster vaccination group had a lower OR for seroprotection compared to the single vaccination group. Particularly, the decrease in OR of the

Table 1 Baseline characteristics of the study subjects

Characteristics	Study subjects *n* (%) All ($N = 89$)	Single group 46	Booster group 43	*p*
Gender				
Male	51 (57 %)	25 (54 %)	26 (60 %)	0.56
Female	38 (43 %)	21 (46 %)	17 (40 %)	
Age at vaccination (years ± SD)	43.9	45.3 (26–73)	42.4 (21–72)	0.29
Immunosuppressive therapy				
Healthy control	11 (12 %)	7 (15 %)	4 (1 %)	
AZA or 6MP	29 (33 %)	14 (30 %)	15 (35 %)	0.82
IFX or ADA	21 (24 %)	10 (22 %)	11 (26 %)	
IFX/ADA and AZA/6MP	28 (31 %)	15 (33 %)	13 (30 %)	
Disease duration (years ± SD)	9.37	8.8 (1–30)	10.0 (1–27)	0.76
Disease				
Crohn's disease	38 (43 %)	20 (22 %)	18 (20 %)	0.77
Ulcerative colitis	33 (37 %)	15 (17 %)	18 (20 %)	
Intestinal Behçet disease	7 (7 %)	4 (4 %)	3 (3 %)	
Disease activity				
HBI (CD)	4.03	3.61 (1–7)	4.47 (1–12)	0.66
Partial Mayo score (UC)	3.12	2.44 (0–8)	3.72 (0–10)	0.19

Data are expressed as no. (%) of patients, unless otherwise indicated

CD Crohn's disease, *UC* ulcerative colitis, *IFX* infliximab, *ADA* adalimumab, *AZA* azathioprine, *6MP* 6-mercaptopurine, *HBI* Harvey–Bradshaw index

booster group was marginally significant for the H1N1 strain (OR 0.34, $p = 0.05$; Table 4). Combination therapy with AZA/6MP and IFX/ADA did not result in a lower OR compared to monotherapy with AZA, 6MP, IFX, or ADA. A higher pre-vaccination titer (≥1:40) was significantly associated with a sufficient seroprotection rate after vaccination for the H1N1 strain (OR 11.93, $p = 0.03$).

Adverse events

There were no severe side effects such as fatalities or anaphylactic shock after vaccination. In the medical records, 4 patients in the single vaccination group and 4 patients in the booster vaccination group complained of pain with swelling at the site of the subcutaneous injection. The second booster vaccination did not result in additional adverse events.

Discussion

The influenza virus infection is an annual issue, and many people receive influenza vaccinations annually. The global H1N1 pandemic and its mortality in 2009 still remains in our memory, and the elderly and children are at high risk for the severe influenza infection [20]. Patients administered immunosuppressive agents are also at a high risk. Recent developments in immunomodulators or anti-TNF-α agents provide better prognoses for IBD patients, and several new immunomodulatory drugs, including biologics for individual target molecules, are under development in clinical trials [21]. Several guidelines recommend the annual influenza vaccination for IBD patients, especially those treated with immunosuppressive drugs, steroids, immunomodulators, or anti-TNF-α agents [26]. Educational intervention is effective for increasing the rate of vaccination [27], and the influenza vaccination is not associated with IBD flares [28].

However, some investigations reported an insufficient immune response to the influenza H1N1 vaccination in IBD patients treated with immunosuppressive drugs [29, 30]. For the first time, we also report an inhibited immune response to some strains of the trivalent influenza vaccination in adult IBD patients treated with IFX and/or immunomodulators [10]. Further investigations are needed to establish the appropriate influenza vaccination program for IBD patients taking immunosuppressive agents.

Lu et al. [11] reported that the trivalent influenza vaccination produces a high prevalence of seroprotection in pediatric IBD patients, particularly against A strains. IBD patients <8 years old received two booster vaccinations in that study. The proportion of seroprotected pediatric IBD patients and GMTs at post-vaccination was similar between the non-immunosuppressed therapy groups and the immunosuppressed therapy groups for all three strains. Regarding the other inactivated vaccine, the HBV vaccine, 76 % pediatric IBD patients who had an insufficient immune response after the first vaccination had an anamnestic response after the second booster vaccination [16]. Therefore, we conducted the present prospective

Table 2 Changes in the parameters of immunogenicity for the 3 strains of the trivalent influenza vaccine during the study period

IBD	Geometric mean titer[a]			Fold rise[a]	Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	S1/S0 for single S2/S0 for booster	Before vaccination (S0)	After vaccination[#]	After season (S3)
H1N1							
Single group	14	86	32	6.35***	8 (21)	33 (85)	27 (55)
Booster group	13	53	27	4.22***	8 (21)	25 (64)	20 (46)
p	0.98	0.09	0.54	0.41	1.00	0.04	0.35
H3N2							
Single group	16	81	57	4.95***	9 (23)	32 (82)	28 (72)
Booster group	20	77	50	3.86***	10 (26)	30 (77)	29 (74)
p	0.29	0.99	0.93	0.37	0.79	0.57	0.80
B							
Single group	68	169	116	2.48***	32 (82)	39 (100)	38 (97)
Booster group	96	169	120	1.77***	39 (100)	39 (100)	39 (100)
p	0.10	0.94	0.90	0.08	0.006	NA	0.31
Healthy control							
H1N1							
Single group	24	65	32	2.69*	4 (57)	6 (86)	5 (71)
Booster group	26	57	48	2.00*	3 (75)	4 (100)	3 (75)
p	0.77	0.92	0.68	0.77	0.55	0.43	0.90
H3N2							
Single group	16	40	24	2.44**	1 (14)	5 (71)	2 (29)
Booster group	28	57	28	2.00*	2 (50)	4 (100)	2 (50)
p	0.16	0.36	0.60	0.77	0.20	0.24	0.48
B							
Single group	98	160	108	1.64*	7 (100)	7 (100)	7 (100)
Booster group	95	160	80	1.68*	4 (100)	4 (100)	3 (75)
p	1.00	1.00	1.00	1.00	NA	NA	0.17

NA not applicable

[#] GMT after 1 vaccination (S1) for once group and GMT after 2 vaccinations (S2) for booster group

* $p < 0.1$, ** $p < 0.05$, *** $p < 0.0001$

[a] Wilcoxon signed-rank test for intracategory comparisons, and either the Wilcoxon rank-sum test or the Kruskal–Wallis test for intercategory comparisons

[b] Seroprotection rate (post-vaccination titer ≥1:40). $\chi 2$ test between 2 categories

randomized controlled study to evaluate the second booster of the trivalent influenza vaccination in adult IBD patients treated with immunosuppressive agents.

Our findings indicate that the booster of the trivalent influenza vaccination does not improve the immune responses to the 3 strains in adult IBD patients who are treated with immunomodulators and/or anti-TNF-α agents. Of note, the second booster of the influenza vaccination did not result in an additional immune response in patients who had an insufficient immune response in the present study (Fig. 2c). Only the single vaccination responded enough to meet the international licensing criteria of the European Agency for the Evaluation of Medical Products. In this study, the seroprotection rate after the first vaccination was high (H1N1: 85 %, H3N2: 82 %, B: 100 %) compared to our previous study (H1N1: 81 %, H3N2: 61 %, B: 86 %) [10]. This good reaction may be the reason for the low increase in antibody titers after the second vaccination. Moreover, when comparing the pre-vaccination titers of each strain between the present study and our previous study, a lower pre-vaccination titer (≤1:10) was observed for H1N1 in 37 and 60 % of participants, for H3N2 in 8 and 60 % of participants, and for B in 0 and 30 % of participants in the present and previous studies, respectively. As a higher pre-vaccination titer will yield a better immunoresponse after a single vaccination, good results were obtained in the present study after single injection [31]. Comparison with healthy controls also helped to understand the immunological status in IBD patients that was not suppressed in this series. Furthermore, a history of

Table 3 Stratified immunogenicity analyses of the 3 strains of the trivalent influenza vaccine according to the type of immunosuppressive treatment or pre-vaccination titer during the study period

	Influenza A (H1N1)					
	Geometric mean titer[a]			Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	Before vaccination (S0)	After vaccination[#]	After season (S3)
Single group						
Treatment						
Healthy control	24	66	36	4 (57)	6 (86)	5 (71)
AZA or 6MP	14	76	46	2 (14)	10 (71)	10 (71)
IFX or ADA	9	98	20	2 (20)	10 (100)	3 (30)
AZA/6MP and IFX/ADA	17	88	32	4 (27)	13 (87)	8 (53)
p	0.21	0.81	0.30	0.19	0.29	0.19
Pre-vaccination titer						
<1:10	5	108	18	0	14 (86)	5 (31)
1:10–1:20	14	54	32	0	13 (72)	10 (56)
≥1:40	71	120	71	12 (100)	12 (100)	11 (92)
p	<0.0001	0.03	0.003	<0.0001	0.11	0.006
Booster group						
Treatment						
Healthy control	28	57	48	3 (75)	4 (100)	3 (75)
AZA or 6MP	11	61	24	2 (13)	10 (67)	6 (40)
IFX or ADA	13	45	24	2 (18)	8 (73)	5 (45)
AZA/6MP and IFX/ADA	14	52	34	4 (31)	7 (54)	7 (54)
p	0.51	0.98	0.62	0.08	0.37	0.68
Pre-vaccination titer						
<1:10	5	34	16	0	8 (47)	5 (29)
1:10–1:20	15	61	35	0	11 (73)	7 (47)
≥1:40	55	91	55	11 (100)	10 (91)	9 (82)
p	<0.0001	0.10	0.008	<0.0001	0.04	0.02
	Influenza A (H3N2)					
	Geometric mean titer[a]			Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	Before vaccination (S0)	After vaccination[#]	After season (S3)
Single group						
Treatment						
Healthy control	16	40	24	1 (14)	5 (71)	2 (26)
AZA or 6MP	19	93	84	4 (29)	11 (76)	12 (86)
IFX or ADA	20	92	49	2 (20)	9 (90)	8 (80)
AZA/6MP and IFX/ADA	13	66	44	3 (20)	12 (80)	8 (53)
p	0.33	0.52	0.08	0.88	0.81	0.03
Pre-vaccination titer						
<1:10	5	23	23	0	2 (40)	1 (20)
1:10–1:20	14	75	45	0	25 (81)	19 (61)
≥1:40	49	121	106	10 (100)	10 (100)	10 (100)
p	<0.0001	0.02	0.02	<0.0001	0.02	0.006
Booster group						
Treatment						
Healthy control	28	57	28	2 (50)	4 (100)	2 (50)

Table 3 continued

	Influenza A (H3N2)					
	Geometric mean titer[a]			Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	Before vaccination (S0)	After vaccination[#]	After season (S3)
AZA or 6MP	21	101	55	5 (33)	12 (80)	11 (73)
IFX or ADA	26	71	58	3 (27)	9 (82)	10 (91)
AZA/6MP and IFX/ADA	15	61	40	2 (15)	9 (69)	8 (62)
p	0.18	0.51	0.31	0.53	0.60	0.30
Pre-vaccination titer						
<1:10	5	20	28	0	1 (50)	1 (50)
1:10–1:20	15	63	37	0	21 (72)	18 (62)
≥1:40	57	143	95	12 (100)	12 (100)	12 (100)
p	<0.0001	0.01	0.004	<0.0001	0.08	0.04
	Influenza B					
	Geometric mean titer[a]			Seroprotection rate (≥1:40), *n* (%)[b]		
	Before vaccination (S0)	After vaccination[#]	After season (S3)	Before vaccination (S0)	After vaccination[#]	After season (S3)
Single group						
Treatment						
Healthy control	98	160	108	7 (100)	7 (100)	7 (100)
AZA or 6MP	57	152	125	10 (71)	14 (100)	14 (100)
IFX or ADA	86	139	98	10 (100)	10 (100)	10 (100)
AZA/6MP and IFX/ADA	70	211	121	12 (80)	15 (100)	14 (93)
p	0.47	0.37	0.69	0.15	NA	0.55
Pre-vaccination titer						
<1:10						
1:10–1:20	18	98	88	0	7 (100)	9 (100)
≥1:40	92	184	120	39 (100)	39 (100)	3 (97)
p	<0.0001	0.02	0.17	<0.0001	NA	0.67
Booster group						
Treatment						
Healthy control	95	160	80	4 (100)	4 (100)	3 (75)
AZA or 6MP	96	175	121	15 (100)	15 (100)	15 (100)
IFX or ADA	117	132	132	11 (100)	11 (100)	11 (100)
AZA/6MP and IFX/ADA	80	198	110	13 (100)	13 (100)	13 (100)
p	0.78	0.64	0.98	NA	NA	0.02
Pre-vaccination titer						
<1:10						
1:10–1:20						
≥1:40	96	168	116	43 (100)	43 (100)	42 (98)

NA not applicable, *IFX* infliximab, *ADA* adalimumab, *AZA* azathioprine, *6MP* 6-mercaptopurine

[#] GMT after 1 vaccination (S1) for one group and GMT after second vaccination (S2) for booster group

[a] Wilcoxon signed-rank test for intracategory comparisons, and either the Wilcoxon rank-sum test or the Kruskal–Wallis test for intercategory comparisons

[b] Seroprotection rate (post-vaccination titer ≥1:40). The Mantel-extension method for trend test among 3 categories

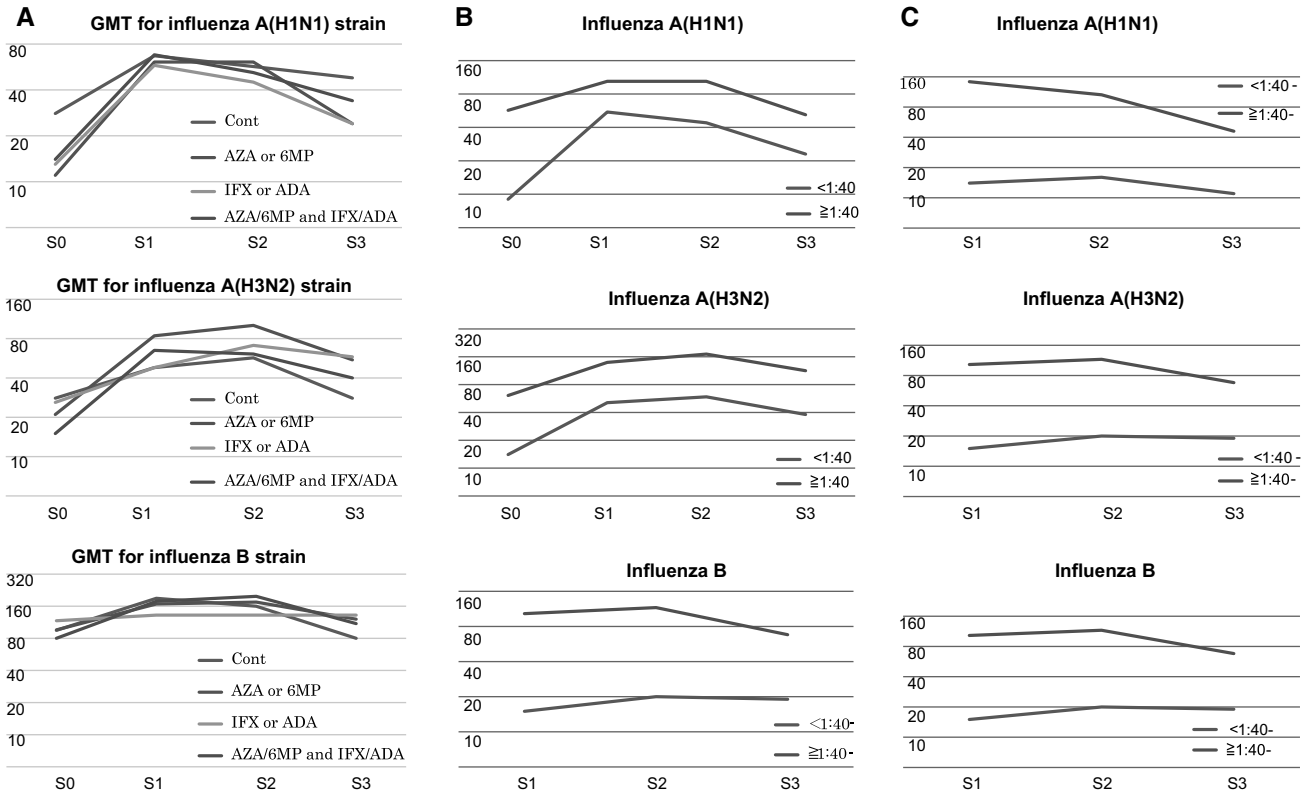

Fig. 2 **a** The change in the geometric mean titers for the 3 strains according to the immunosuppressive treatment in the booster vaccination group involving IBD patients and controls. **b** The changing process of geometric mean titers for the 3 strains according to the pre-vaccination titer in the booster vaccination group. **c** The changing process of geometric mean titer for the 3 strains according to the immune response at 3 weeks after the first dose (S1) after the first vaccination in the booster vaccination group

influenza infection or vaccination influences the immunoresponse for each strain. In Japan, vaccination for influenza has been performed for the same H1N1 strain annually after 2011. However, a difference was noted for strain B between 2010 and 2011 (Victoria lineage), and 2012 (Yamagata lineage) based on the estimation of the influenza epidemic.

A randomized study in children showed that the second booster of the influenza vaccination was effective for improving the seroprotection rate [32]. In contrast, the booster vaccination did not provide an anamnestic immune response in the elderly [32–34]. We reported that the second booster vaccination was not as effective in adults with severe motor and intellectual disabilities [35]. Thus, we expected that the most important factor of the immune response for the second booster vaccination was age. Humoral immune responses develop with increasing age, supporting the notion of broadening of immune responses and affinity maturation of the antibodies that are produced [33, 34].

There were limitations to the present study. The number of participants was small. When participant recruitment was initiated (November 2012), the influenza vaccination period had already begun in Japan. Hence, many IBD patients in our hospital may have been administered the vaccination in other hospitals or clinics. Therefore, we could not meet our target for the number of patients recruited. However, if the number of participants were increased, the results would not be so different from our current results according to the statistical analysis. The immune response to influenza strain B is usually less seroprotected compared to strain A [11]. Yet, the pre-vaccination titer of strain B was extremely high in our study. Pre-existing antibody titers provide a substantial effect on immune response [35]. Our study also showed that higher pre-vaccination titers to the H1N1 strain were associated with a sufficient immune response for the influenza vaccination (Fig. 2a; Table 4).

Immune response is different from the incidence rate of influenza. The approaches based on $CD4^+$ or $CD8^+$ T cells specific to the conserved viral core protein epitopes correlated with the cross-reactive cellular immune responses, not the strain-matched B cell, which may make the development of a novel influenza vaccine possible [35, 36].

In conclusion, this is the first prospective randomized controlled study to investigate the efficacy of a second booster vaccination on the immune response in adult IBD

Table 4 Multivariate analysis of the associated factors for a sufficient seroprotection rate after vaccination

	Influenza A (H1N1) OR (95 % CI)	*p*	Influenza A (H3N2) OR (95 % CI)	*p*
One S1	1		1	
Booster S2	0.34 (0.11–1.01)	0.05	0.68 (0.22–2.17)	0.52
Immunosuppressive therapy				
Healthy control	1		1	
AZA or 6MP	0.58 (0.05–6.41)	0.65	1.11 (0.16–7.74)	0.92
IFX or ADA	1.64 (0.12–22.05)	0.71	1.60 (0.20–12.58)	0.66
AZA/6MP and IFX/ADA	0.49 (0.04–5.38)	0.56	1.12 (0.17–7.60)	0.91
Pre-vaccination titer				
<1:10	1		1	
1:10–1:20	1.34 (0.44–4.09)	0.61	4.44 (0.80–24.77)	0.09
≥1:40	11.93 (1.30–109.19)	0.03	Not applicable	

Model included all variables in this table

IFX infliximab, *ADA* adalimumab, *AZA* azathioprine, *6MP* 6-mercaptopurine

Logistic regression model: *CI* confidence interval, *OR* odds ratio

patients taking immunosuppressive agents. The second booster of the trivalent influenza vaccination did not improve the immune response of adult IBD patients treated with immunomodulators and/or anti-TNF-α agents. The booster influenza vaccination does not appear to be necessary in adult IBD patients and healthy adults. With regard to the trends of immunosuppressive therapy for IBD patients, further investigations are essential for establishing an appropriate influenza vaccination strategy in high-risk IBD patients.

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Conflict of interest Kenji Watanabe lectured for AbbVie Japan, Mitsubishi Tanabe Pharma Corporation, Eisai, and has received unrestricted research grants from AbbVie Japan, Mitsubishi Tanabe Pharma Corporation, and Eisai.

References

1. Abraham C, Cho JH. Functional consequences of NOD2 (CARD15) mutations. Inflamm Bowel Dis. 2006;12:641–50.
2. Aberra FN, Lichtenstein GR. Methods to avoid infections in patients with inflammatory bowel disease. Inflamm Bowel Dis. 2005;11:685–95.
3. Lichtenstein GR, Feagan BG, Cohen RD, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. Clin Gastroenterol Hepatol. 2006;4:621–30.
4. Viget N, Vernier-Massouille G, Salmon-Ceron D, et al. Opportunistic infections in patients with inflammatory bowel disease: prevention and diagnosis. Gut. 2008;57:549–58.
5. Naganuma M, Kunisaki R, Yoshimura N, et al. A prospective analysis of the incidence of and risk factors for opportunistic infections in patients with inflammatory bowel disease. J Gastroenterol. 2013;48:595–600.
6. Ueno F, Matsui T, Matsumoto T, et al. Evidence-based clinical practice guidelines for Crohn's disease, integrated with formal consensus of experts in Japan. J Gastroenterol. 2013;48:31–72.
7. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA. 2003;289:179–86.
8. Thompson WW, Shay DK, Weintraub E, et al. Influenza-associated hospitalizations in the United States. JAMA. 2004;292:1333–40.
9. Cohn AC, MacNeil JR, Clark TA, et al. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2013;62:1–28.
10. Hagihara Y, Ohfuji S, Watanabe K, et al. Infliximab and/or immunomodulators inhibit immune responses to trivalent influenza vaccination in adults with inflammatory bowel disease. J Crohns Colitis. 2014;8:223–33.
11. Lu Y, Jacobson DL, Ashworth LA, et al. Immune response to influenza vaccine in children with inflammatory bowel disease. Am J Gastroenterol. 2009;104:444–53.
12. Kathleen M, Neuzil LAJ, Nelson J, Klimov A, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-native 5-8-year-old children. J Infect Dis. 2006;194:1032–9.
13. Englund JA, Walter EB, Fairchok MP, et al. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. Pediatrics. 2005;115:1039–47.
14. Opinion of the EMEA on the potential risk associated with medicinal products in relation to bovine spongiform encephalopathy (BSE) (16 April 1996) and report from the Committee for Proprietary Medicinal Products (CPMP) on the 'Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathies via medicinal products' (15 April 1997). Adverse Drug React Toxicol Rev 1997;16:113–21.
15. Zhu FC, Wang H, Fang HH, et al. A novel influenza A (H1N1) vaccine in various age groups. N Engl J Med. 2009;361:2414–23.
16. Moses J, Alkhouri N, Shannon A, et al. Hepatitis B immunity and response to booster vaccination in children with inflammatory bowel disease treated with infliximab. Am J Gastroenterol. 2012;107:133–8.
17. Saad CG, Borba EF, Aikawa NE, et al. Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases. Ann Rheum Dis. 2011;70:1068–73.
18. Esteve Comas M, Loras Alastruey C, Fernandez-Bañares F. How do we manage vaccinations in patients with inflammatory bowel disease? Dig Dis. 2009;27:370–4.
19. Ohfuji S, Fukushima W, Deguchi M, et al. Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine among pregnant

women: lowered antibody response by prior seasonal vaccination. J Infect Dis. 2011;203:1301–8.
20. Matias G, Taylor R, Haguinet F, et al. Estimates of mortality attributable to influenza and RSV in the United States during 1997–2009 by influenza type or subtype, age, cause of death, and risk status. Influenza Other Respir Viruses. 2014;8:507–15.
21. Danese S. New therapies for inflammatory bowel disease: from the bench to the bedside. Gut. 2012;61:918–32.
22. Viget N, Vernier-Massouille G, Salmon-Ceron D, et al. Opportunistic infections in patients with inflammatory bowel disease: prevention and diagnosis. Gut. 2008;57:549–58.
23. Parker S, Chambers White L, Spangler C, et al. A quality improvement project significantly increased the vaccination rate for immunosuppressed patients with IBD. Inflamm Bowel Dis. 2013;19:1809–14.
24. Rahier JF, Papay P, Salleron J, et al. H1N1 vaccines in a large observational cohort of patients with inflammatory bowel disease treated with immunomodulators and biological therapy. Gut. 2011;60:456–62.
25. Andrisani G, Frasca D, Romero M, et al. Immune response to influenza A/H1N1 vaccine in inflammatory bowel disease patients treated with anti TNF-α agents: effects of combined therapy with immunosuppressants. J Crohns Colitis. 2013;7:301–7.
26. Cullen G, Bader C, Korzenik JR, Sands BE. Serological response to the 2009 H1N1 influenza vaccination in patients with inflammatory bowel disease. Gut. 2012;61:385–91.
27. Gross PA, Weksler ME, Quinnan GV, Douglas RG, Gaerlan PF, Denning CR. Immunization of elderly people with two doses of influenza vaccine. J Clin Microbiol. 1987;25:1763–5.
28. Feery BJ, Cheyne IM, Hampson AW, et al. Antibody response to one and two doses of influenza virus subunit vaccine. Med J Aust. 1976;1(186):188–9.
29. Levine M, Beattie BL, McLean DM. Comparison of one- and two-dose regimens of influenza vaccine for elderly men. CMAJ. 1987;137:722–6.
30. Hara M, Hanaoka T, Mizushima T, et al. Diminished immunogenicity to pandemic H1N1 2009 influenza vaccine in subjects with severe motor and intellectual disability. Vaccine. 2011;29:8323–9.
31. Hobson D, Baker FA, Curry RL. Effects of influenza vaccines in stimulating antibody in volunteers with prior immunity. Lancet. 1973;2:155–6.
32. Davenport FM, Hennessy AV, Francis T. Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus. J Exp Med. 1953;98:641–56.
33. Epstein SL, Price GE. Cross-protective immunity to influenza A viruses. Expert Rev Vaccines. 2010;9:1325–41.
34. Kobayashi M, Ohfuji S, Fukushima W, et al. Immunogenicity and reactogenicity of a monovalent inactivated 2009 influenza A vaccine in adolescents: with special reference to pre-existing antibody. J Pediatr. 2012;160:632–7.
35. Sridhar S, Begom S, Bermingham A, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. Nat Med. 2013;19:1305–12.
36. Bonduelle O, Yahia N, Siberil S, et al. Longitudinal and integrative biomodeling of effector and memory immune compartments after inactivated influenza vaccination. J Immunol. 2013;191:623–31.

INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Safety of a Pandemic Influenza Vaccine and the Immune Response in Patients with Duchenne Muscular Dystrophy

Tomoko Saito[1,2], Satoko Ohfuji[1], Tsuyoshi Matsumura[2], Toshio Saito[2], Kazuhiro Maeda[3], Akiko Maeda[1], Wakaba Fukushima[1], Harutoshi Fujimura[2], Saburo Sakoda[2] and Yoshio Hirota[1]

Abstract

Objective To examine the safety of and immune response to the influenza A(H1N1)pdm09 vaccine in patients with Duchenne muscular dystrophy (DMD).

Methods Forty-four non-ambulatory patients with DMD hospitalized in a muscle disease ward and 41 healthy healthcare workers each received one dose of the influenza A(H1N1)pdm09 vaccine. Serum samples were collected before and four weeks after vaccination to measure the hemagglutinin inhibition antibody titers.

Results No severe adverse events were noted in any of the subjects. The immune responses of the patients were comparable to those of the healthcare workers. Among the patients, tube feeding and a lower total protein level in the serum were identified to be significantly associated with a lower immune response.

Conclusion A single dose of the vaccine was found to be safe and induced an optimal level of immunity in the DMD patients. The nutritional status may be associated with the immune response in patients with DMD.

Key words: Duchenne muscular dystrophy, influenza vaccine, safety, immune response

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Introduction

Duchenne muscular dystrophy (DMD) is an inherited myogenic disorder characterized by progressive skeletal muscle involvement in which weakness of the respiratory muscles, distortion of the thorax and inability to perform postural changes result in the retention of secretions and chronic microatelectasis. In association with pulmonary dysfunction, respiratory infections, including influenza, can cause severe complications that further weaken the respiratory function, necessitating admission to an intensive care unit and potentially causing death (1, 2). Therefore, preventing respiratory infections is a matter of clinical importance. Although recent guidelines for the treatment of DMD recommend annual influenza vaccination (3, 4), there are currently no reports regarding the immune response in patients treated with these vaccines. Another concern is whether disease-related conditions, such as the patient's physical condition and nutritional status, are associated with the antibody response in cases of DMD.

In the present study, we investigated whether influenza vaccination is safe and immunogenic in patients with DMD as compared to that observed in healthy healthcare workers and identified the factors affecting the immune response in DMD patients.

Materials and Methods

Subjects

We invited 46 inpatients with DMD treated at National Hospital Organization Toneyama National Hospital from October 21 to 30, 2009, of whom 44 agreed to participate. All

[1]Department of Public Health, Osaka City University Graduate School of Medicine, Japan, [2]Department of Neurology, National Hospital Organization Toneyama National Hospital, Japan and [3]Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University, Japan
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Correspondence to Dr. Tomoko Saito, tosaito@toneyama.go.jp

subjects were non-ambulatory, including 33 (75%) patients from the long-term care unit and 11 (25%) short-term inpatients. During the same period, 41 healthcare workers employed by the same hospital were also recruited to participate in the study as a control group. None of the subjects met the exclusion criteria, including prior episodes of influenza A(H1N1)pdm09 infection, acute febrile illnesses at the time of vaccination, history of anaphylaxis resulting from the vaccine components or other conditions making it inappropriate to undergo vaccination. Written informed consent was obtained from each participant or their guardian if younger than 20 years of age at the time of recruitment. The study protocol was reviewed and approved by the ethics committees of Osaka City University Graduate School of Medicine and Toneyama National Hospital.

Data collection

Prior to vaccination, all participants completed a self-administered questionnaire regarding sex, date of birth, height, weight and comorbid diseases. In addition, clinical information was extracted from the patients' medical records, including medications, ejection fraction of the left ventricle (EF) within the last six months, activities of daily living (ADLs), use of mechanical ventilation and levels of total protein, albumin, hemoglobin and hematocrit on routine laboratory tests.

Vaccine

A monovalent, unadjuvanted, inactivated, split influenza A (H1N1)pdm09 vaccine (Lot. HP01A; BIKEN) was used. All participants received a subcutaneous injection of the vaccine at a dose of 0.5 mL containing 15 μg of hemagglutinin antigens and 0.0008% thimerosal.

Assessment of adverse reactions

All vaccinated subjects recorded solicited local and systemic reactions occurring within 48 hours after vaccination using a self-administered questionnaire. For patients who were unable to independently fill in the questionnaire, nurses completed the form based on a direct interview and observation. Local reactions included redness, swelling, induration, itching and pain at the injection site. Systemic reactions included fever (axillary temperature ≥37.5℃), malaise, myalgia, headache and rashes.

Measurement of the antibody titer

Blood samples were collected 0-2 days before and 28-30 days after vaccination. The serum was stored at -20℃ until all samples were assayed at the same time in July 2010. The titer of serum antibodies to hemagglutinin was measured using the standard microtiter HAI method (5). All samples were tested at the laboratory of the Research Foundation for Microbial Diseases of Osaka University.

Statistical analysis

For comparisons of the baseline variables, adverse reactions and antibody responses between the subject groups (patients vs. healthcare workers), the Wilcoxon rank-sum test was used for continuous variables and the chi-square test, Fisher's exact test and Mantel-extension method were used for categorical variables.

Categorical variables included the pre-vaccination titer (<1:10, 1:10-1:20, and ≥1:40), ADLs (wheelchair use or bedridden status) and mechanical ventilation (none, noninvasive positive-pressure ventilation (NPPV) or tracheal positive-pressure ventilation (TPPV)). The immunogenicity endpoints were determined based on conventional international criteria, as follows: geometric mean titer (GMT), fold rise, seroprotection proportion (post-vaccination titer ≥1:40) and seroresponse proportion (fold rise ≥4) (6, 7). A titer of <1:10 was defined as 1:5 for the calculations. Reciprocal antibody titers were analyzed after logarithmic transformation. The results are presented in the original scale by calculating the antilogarithm.

We evaluated the independent effects of several factors on immunogenicity solely in the patient group, then calculated the odds ratio (OR) and 95% confidence interval (CI) using logistic regression models. Since only 44 patients were enrolled, care was taken to select explanatory variables for the multivariate models. In the first multivariate model (model 1), we controlled for age and pre-vaccination titer, which have been inconsistently reported to be related to immunogenicity from influenza vaccinations (8-10). In order to obtain meaningful calculation results, we combined the pre-vaccination subcategory titers 1:10-1:20 and ≥1:40 into one category of ≥1:10. In model 2, in order to identify additional potential confounders, we used a stepwise regression model (significance level for entry into the model =0.15), which resulted in the feeding method being included.

Two-sided p values less than 0.05 were considered to be statistically significant. All analyses were performed using the SAS software package, version 9.1 (SAS Institute, Inc., Cary, USA).

Results

Study subjects

Table 1 summarizes the baseline characteristics of the subjects. Most of the patients were in an advanced stage, with a high age, low cardiopulmonary function and low level of ADLs. None of the patients were currently receiving oral steroid therapy, although this therapy is now the standard treatment worldwide for young patients with DMD (11).

Vaccine safety

Solicited adverse reactions to the vaccine among the patients and healthcare workers are shown in Table 2. Both local and systemic reactions were less frequent in the patients. All symptoms were mild, and none of the affected subjects required medical treatment.

Table 1. Baseline Characteristics of the Study Subjects (n=85).

Characteristics	Total (n=85)	Patients (n=44)	Healthcare workers (n=41)	p value*
(Comparison between patients and healthcare workers)				
Sex: male, *n* (%)	62 (73)	44 (100)	18 (44)	0.001
Age (years)				
mean (SD)	35.8 (11.1)	30.9 (8.6)	41.2 (11.1)	<0.001
median (range)	33.0 (17-62)	31.3 (17-47)	43.0 (23-62)	
Body mass index (kg/m^2)				
mean (SD)	17.4 (4.5)	13.7 (2.4)	21.4 (2.4)	<0.001
median (range)	17.6 (10.2-30.5)	13.3 (10.2-19.5)	21.2 (17.1-21.2)	
Prevaccination titer				
<1:10, *n* (%)	57 (67)	31 (70)	26 (63)	0.857
1:10-1:20, *n* (%)	24 (28)	11 (25)	13 (32)	
≥1:40, *n* (%)	4 (5)	2 (5)	2 (5)	
Underling disease				
diabetes mellitus, *n* (%)	1 (1)	1 (2)	0 0	1.000
atopic dermatitis, *n* (%)	4 (5)	3 (7)	1 (2)	0.617
(Specific factors in patients)				
EF (%), *mean (SD)*		36.7 (15.3)		
Activity of daily living				
wheelchair user, *n* (%)		17 (39)		
bedridden, *n* (%)		27 (61)		
Respiratory status				
none, *n* (%)		2 (5)		
NPPV, *n* (%)		16 (36)		
TPPV, *n* (%)		26 (59)		
Feeding method				
tube feeding (-), *n* (%)		20 (45)		
tube feeding (+) **, *n* (%)		24 (55)		
Albumin (g/dl), *mean (SD)*		3.9 (0.5)		
Total Protein (g/dl), *mean (SD)*		7.1 (0.5)		
Hemoglobin (g/dl), *mean (SD)*		12.8 (1.5)		
Hematocrit (%), *mean (SD)*		38.1 (4.5)		

EF: ejection fraction of the left ventricle, NPPV: non-invasive positive pressure ventilation, TPPV: Tracheal positive pressure ventilation

* Wilcoxon rank sum test, χ^2 test, Fisher's exact test or Mantel extension method for trend tests

** Tube feeding (+): gastrostomy feeding (n=5), nasal or oral tube feeding (n=19)

Table 2. Local and Systemic Reactions to the Vaccine.

	Patients (n=44)		Healthcare workers (n=41)		
Symptom	n	%	n	%	p value*
Local reactions					
Total	14	32	21	51	0.071
Redness	12	27	17	41	0.170
Swelling	4	9	8	20	0.171
Induration	0	0	7	17	0.005
Itching	0	0	8	20	0.002
Pain	2	5	13	32	0.001
Systemic reactions					
Total	3	7	12	29	0.007
Fever (>37.5˚C)	2	5	4	10	0.423
Malaise	0	0	8	20	0.002
Myalgia	0	0	2	5	0.230
Headache	0	0	2	5	0.230
Rash	1	2	0	0	1.000

* χ^2 test or Fisher's exact test

Immune response

The results for the antibody response in relation to the background factors are shown in Table 3. The only identified significant factor was the pre-vaccination titer, as a higher pre-titer value was associated with a greater post-vaccination GMT, lower seroresponse proportion and higher seroprotection proportion. There were no significant differences in any of the endpoints of immunogenicity between the subject groups. In the logistic regression analysis, the OR after adjustment for age and pre-vaccination titer in the patients as compared to the healthcare workers was 1.71 (95%CI: 0.50-5.87) for the seroresponse proportion and 0.88 (0.29-2.63) for the seroprotection proportion, neither of which were statistically significant.

Figure shows the pre- and post-vaccination GMTs in the patients based on several predictors. In a comparison of the fold rise between each factor, we found that the oral-fed patients exhibited better fold rise values than the tube-fed patients (16 vs. 7, p=0.047). We also examined the effects of disease-related factors on the seroresponse and seroprotection proportions, with the results shown in Tables 4 and 5. An older age was suggested to have a relationship with a greater seroresponse in model 2. Furthermore, the tube-fed patients demonstrated a decreased OR for the seroresponse proportion compared to the oral-fed patients, and a higher total protein level was found to be significantly associated with a higher seroprotection proportion. Variables of the functional status, such as EF, ADLs and the respiratory condition, were not related to the immune response.

Table 3. Immunogenicity to the Vaccine Based on the Background Factors (n=85).

		GMT		Fold rise	Seroresponse	Seroprotection
	N	S0	S1		n (%)	n (%)
Entire sample	85	7	72	9.7	68 (80)	61 (72)
Sex						
Male	62	8	79	10.5	51 (82)	45 (73)
Female	23	7	56	8.0	17 (74)	16 (70)
p value		0.488	0.274	0.428	0.381	0.785
Age (years)						
<34.5	42	8	74	9.6	34 (81)	31 (74)
≥34.5	43	7	70	9.9	34 (79)	30 (70)
p value		0.589	0.686	0.993	0.829	0.681
Body mass index (kg/m²)						
<18.5	45	7	70	9.6	37 (82)	31 (69)
≥18.5	40	8	75	9.8	31 (78)	30 (75)
p value		0.494	0.947	1.000	0.589	0.535
Pre-vaccination titer						
<1:10	57	5	51	10.2	46 (81)	35 (61)
1:10-1:20	24	12	127	10.4	21 (88)	22 (92)
≥1:40	4	95	320	3.4	1 (25)	4 (100)
p value		<0.001	0.002	0.222	0.035	0.010
Subject group						
Patients	44	7	75	10.5	37 (84)	31 (70)
Healthcare workers	41	8	69	9.0	31 (76)	30 (73)
p value		0.508	0.631	0.571	0.332	0.782

GMT: geometric mean titer, S0: pre-vaccination, S1: post-vaccination, Fold rise: S1/S0, Seroresponse: S1/S0 ≥4, Seroprotection:S1 ≥1:40

p value: The Wilcoxon rank-sum test or Kruskal-Wallis rank test were used to compare the GMT and fold rise, while the χ^2 test, Fisher's exact test or Mantel extension method were used to compare the seroprotection and seroresponse proportions.

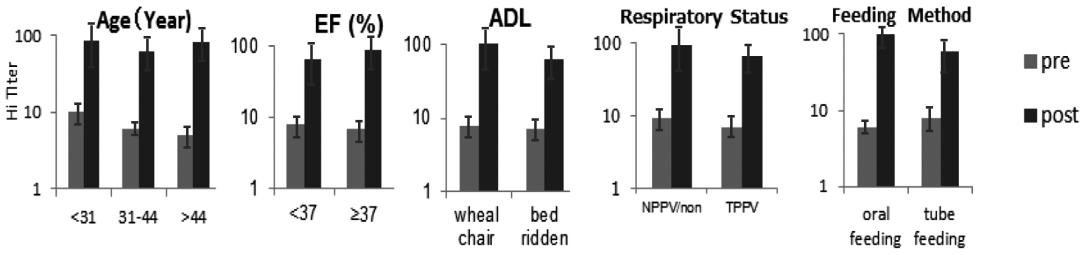

Figure. Pre- and post-vaccination HAI titers in the patients (n=44) based on age and disease-related factors. EF: ejection fraction, ADL: activities of daily living, NPPV: non-invasive intermittent positive pressure ventilation, TPPV: tracheal positive pressure ventilation. Age is presented according to tertile. The EF is presented according to the median. *In a comparison using the Wilcoxon rank-sum test or Kruskal-Wallis rank test, the p values for post-vaccination GMT were not significant, while the p value for the fold rise (post-/pre-vaccination GMT) in the feeding method was 0.047.

Discussion

No harmful adverse effects from vaccination were observed in any of the participants, while the patients with DMD experienced less frequent local and systemic reactions. Information bias derived from the self-administered questionnaire protocol may have been present, as the healthcare workers may have been more sensitive to subtle changes after vaccination. However, the patients showed lower risks for each objective reaction observed by the nurses, including redness, swelling and induration, and the lower frequency of induration was significant. There are likely several modifiers of inflammatory mediators, including sun exposure (12) and immobility, in patients with DMD that may decrease stimulation, although the pathophysiology of skin reactions remains unclear. Nevertheless, the present results are encouraging for both patients and clinicians concerned about risks associated with influenza vaccination.

The immune responses to the influenza vaccine were comparable between the patients with DMD and the health-

Table 4. Associations between Selected Characteristics and the Seroresponse Proportion in the Patients (n=44).

Category	Crude OR (95%CI)	Multivariate model 1* OR (95%CI)	Multivariate model 2* OR (95%CI)
Age			
1 year increased	1.10 (0.98-1.22)	1.14 (0.99-1.22)‡	1.23 (1.02-1.48)†
BMI			
1 kg/m^2 increased	1.38 (0.86-1.17)	1.38 (0.85-2.24)	1.29 (0.79-2.12)
Pre-vaccination titer			
≥1:10/<1:10	1.06 (0.18-6.30)	3.14 (0.36-27.12)	4.59 (0.37-56.70)
EF			
1% increased	1.00 (0.95-1.05)	1.02 (0.96-1.08)	1.02 (0.95-1.10)
Activities of daily living			
bedridden/wheelchair user	2.46 (0.48-12.72)	2.02 (029-13.82)	5.21 (0.53-51.58)
Respiratory status			
TPPV/NPPV or none	2.19 (0.43-11.2)	1.11 (0.14-9.07)	6.81 (0.51-92.28)
Feeding method			
tube feeding (+/-)	0.16 (0.02-1.44)	0.06 (0.01-0.83)†	(identical in model 1)
Albumin			
0.1 g/dL increased	1.00 (0.85-1.18)	1.03 (0.84-1.25)	0.92 (0.73-1.15)
Globulin**			
0.1 g/dL increased	1.10 (0.95-1.28)	1.08 (0.90-1.28)	1.24 (0.99-1.54)
Total protein			
0.1 g/dL increased	1.14 (0.96-1.37)	1.13 (0.93-1.38)	1.18 (0.95-1.49)
Hemoglobin			
0.1 g/dL increased	1.02 (0.97-1.08)	1.08 (0.90-1.28)	1.01 (0.95-1.07)
Hematocrit			
1% increased	1.08 (0.90-1.29)	1.09 (0.90-1.31)	1.01 (0.82-1.25)

Logistic regression model CI: confidence interval, OR: odds ratio, EF: ejection fraction of left ventricle, NPPV: non-invasive positive pressure ventilation, TPPV: tracheal positive pressure ventilation, †p<0.05, ‡ p<0.10

*model 1: adjusted for age and pre-vaccination titer

*model 2: adjusted for all variables in model 1 plus feeding method

** globulin=total protein - albumin

care workers in the present study. The primary factor significantly associated with immunogenicity was the pre-vaccination titer, as a higher pre-vaccination titer was found to be significantly associated with a higher post-vaccination titer, lower seroresponse and higher seroprotection proportion. The inverse association with the seroresponse reflects an effect of "the law of initial values" or "negative feedback" (8, 9). It is important to take the pre-vaccination titer into account when evaluating the immune response to pandemic vaccines. As such studies are often performed during pandemic waves and asymptomatic infections in the study population are inevitable, it is difficult to predict how the immune status prior to vaccination has been modified. We believe that our multivariate analysis including the pre-vaccination titer was adequate to appropriately examine the immune response in this study.

We also found that an increased age was associated with an increased seroresponse in the patient population. Previous studies have reported decreased immune responses in elderly individuals 65 years of age or older (10, 13). However, the mean age of our patients was 30.9 years, with the oldest patient being 47; thus, we cannot simply compare our results to those of other studies. The oldest group in the present study had the lowest pre-GMT and highest post-GMT values (Figure). On the other hand, the EF, ADLs and respiratory status were not significantly associated with the immune response, which indicates that the disease stage or severity is not associated with immunity. Although several specific factors are assumed to be related to long-term survival in DMD patients (14), a superior antibody response in older patients has not been previously reported. Further cell biological and epidemiological investigations of the immune status of long-term survivors with DMD will provide new insight.

The significant OR values for tube feeding and total protein in the present study indicate that the nutritional status is an independent predictor of the antibody response in patients with DMD. Our results are consistent with those of previous studies showing that the nutritional status is associated with immunogenicity in elderly persons (15-17). These findings may help to increase awareness regarding the higher burden of infection in tube-fed patients with DMD.

This study is associated with several limitations. The investigation was conducted in a single hospital and the number of subjects was small; therefore, the study power was limited. In addition, most of the patients were in an advanced stage of disease, which may limit the generalizability

Table 5. Associations between Selected Characteristics and the Seroprotection Proportion in the Patients (n=44).

Category	Crude OR (95%CI)	Multivariate model 1* OR (95%CI)	Multivariate model 2* OR (95%CI)
Age			
1 year increased	0.98 (0.91-1.06)	1.05 (0.95-1.15)	1.05 (0.94-1.16)
BMI			
1 kg/m^2 increased	1.03 (0.78-1.36)	0.84 (0.59-1.21)	0.87 (0.60-1.26)
Pre-vaccination titer			
≥1:10/<1:10	NA	NA	NA
EF			
1% increased	1.01 (0.97-1.06)	1.03 (0.97-1.10)	1.03 (0.97-1.10)
Activities of daily living			
bedridden/wheelchair user	0.62 (0.16-2.44)	1.12 (0.23-5.53)	1.33 (0.25-7.10)
Respiratory status			
TPPV/NPPV or none	0.87 (0.23-3.26)	2.73 (0.39-18.92)	5.45 (0.51-58.35)
Feeding method			
tube feeding (+/-)	0.42 (0.11-1.64)	0.35 (0.08-1.62)	(identical in model 1)
Albumin			
0.1 g/dL increased	1.17 (1.00-1.36)†	1.14 (0.95-1.37)‡	1.11 (0.92-1.34)
Globulin**			
0.1 g/dL increased	1.03 (0.93-1.15)	1.07 (0.93-1.24)	1.13 (0.95-1.33)
Total protein			
0.1 g/dL increased	1.24 (1.04-1.47)†	1.28 (1.03-1.60)†	1.45 (1.04-2.01)†
Hemoglobin			
0.1 g/dL increased	1.05 (1.00-1.10)‡	1.05 (0.99-1.10)	1.04 (0.98-1.10)
Hematocrit			
1% increased	1.17 (1.00-1.37)‡	1.16 (0.97-1.40)	1.14 (0.94-1.38)

Logistic regression model CI: confidence interval, OR: odds ratio, EF: ejection fraction of left ventricle, NPPV: non-invasive positive pressure ventilation, TPPV: tracheal positive pressure ventilation, NA: not applicable,
†p<0.05, ‡ p<0.10
*model 1: adjusted for age and pre-vaccination titer
*model 2: adjusted for all variables in model 1 plus feeding method
** globulin=total protein - albumin

of our findings. Furthermore, since none of the study patients were given oral corticosteroids, we were unable to evaluate the influence of immunosuppressive therapy on the efficacy of vaccination. Additional studies with larger cohorts including young patients and long-term survivors are thus needed to thoroughly investigate immunogenicity to influenza vaccination in cases of DMD.

In conclusion, we found that the influenza A(H1N1)pdm 09 vaccine safely induced a good immune response in patients with DMD. Influenza infection is sometimes lethal in DMD patients. The present results provide useful information for preventing influenza infection in patients with DMD.

The authors state that they have no Conflict of Interest (COI).

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References

1. Bach JR, Ishikawa Y, Kim H. Prevention of pulmonary morbidity for patients with Duchenne muscular dystrophy. Chest **112**: 1024-1028, 1997.
2. Finder JD, Birnkrant D, Carl J, et al. Respiratory care of the patient with Duchenne muscular dystrophy: ATS consensus statement. Am J Respir Crit Care Med **170**: 456-465, 2004.
3. Bushby K, Finkel R, Birnkrant DJ, et al; DMD Care Considerations Working Group. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lancet Neurol **9**: 177-189, 2010.
4. Practical guideline for Duchenne muscular dystrophy Working Group. Practical guideline for Duchenne muscular dystrophy 2014. Nankodo, Tokyo, 2014: 81-82 (in Japanese).
5. World Health Organization. WHO manual on animal influenza diagnosis and surveillance [Internet]. [cited 2013 June 17]. Available from: http://whqlibdoc.who.int/hq/2002/who_cds_csr_ncs_2002.5.pdf
6. Committee for Proprietary Medical Products. Note for guidance on harmonization of requirements for influenza vaccines. [Internet]. [cited 2013 June 17]. Available from: http://www.ema.europa.

eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf

7. Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices- United States, 2013-2014: MMWR 62(RR-07): 1-43, 2013.

8. Hobson D, Baker FA, Curry RL. Effect of influenza vaccines in stimulating antibody in volunteers with prior immunity. Lancet **2**: 155-156, 1973.

9. Hirota Y, Kaji M, Ide S, Goto S, Oka T. The hemagglutination inhibition antibody responses to an inactivated influenza vaccine among healthy adults: with special reference to the prevaccination antibody and its interaction with age. Vaccine **14**: 1597-1602, 1996.

10. Beyer WE, Palache AM, Baljet M, Masurel N. Antibody induction by influenza vaccines in the elderly: a review of the literature. Vaccine **7**: 385-394, 1989.

11. Bushby K, Finkel R, Birnkrant DJ, et al; DMD Care Considerations Working Group. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol **9**: 77-93, 2010.

12. Henri P, Lambert A, Philippe E. Laurent Intradermal vaccine delivery: Will new delivery systems transform vaccine administration? Vaccine **26**: 3197-3208, 2008.

13. Zhu FC, Wang H, Fang HH, et al. A novel influenza A (H1N1) vaccine in various age groups. N Engl J Med **361**: 2414-2423, 2009.

14. Ishizaki M, Ueyama H, Masida T, Nishida Y, Imamura S, Ando Y. Two cases of Duchenne muscular dystrophy over 40 years after onset. Rinshou Shinkeigaku (Clin Neurol) **53**: 293-298, 2013 (in Japanese, Abstract in English).

15. Hara M, Tanaka K, Hirota Y. Immune response to influenza vaccine in healthy adults and the elderly: association with nutritional status. Vaccine **23**: 1457-1463, 2005.

16. Hui SL, Chu LW, Peiris JS, Chan KH, Chu D, Tsui W. Immune response to influenza vaccination in community-dwelling Chinese elderly persons. Vaccine **24**: 5371-5380, 2006.

17. Fülöp T Jr, Wagner JR, Khalil A, Weber J, Trottier L, Payette H. Relationship between the response to influenza vaccination and the nutritional status in institutionalized elderly subjects. J Gerontol A Biol Sci Med Sci **54A**: M59-M64, 1999.