Main diseases controlled by vaccination

The *Guidelines* discuss influenza, measles, pertussis, diphtheria, and tuberculosis. Also proposed are draft diagnostic criteria for pertussis based on epidemiological data that factor in the relative increase in the disease among older children and adolescents and DTP-vaccinated children and adults. Although affected older children and adults exhibit prolonged and severe coughing, no characteristic symptoms can be identified in children without a detailed interview. The disease lacks elevated white blood cell and lymphocyte counts. A novel trivalent vaccine for adolescents and adults (Tdap) has been developed in Europe and the USA.

Pathogen resistance in community-acquired childhood respiratory infections

A classification system for *S. pneumoniae* and *H. influenzae* based on the analysis of antibiotic resistance genes is presented.[41] Antimicrobial agents currently used to treat resistant pathogens are listed (note: the antimicrobial susceptibility of *S. pneumoniae* and *H. influenzae* is discussed in Ubukata[37]). Most strains with an ampicillin-MIC ≤ 2 μg/mL are treatable using oral amoxicillin or i.v. ampicillin, but when therapy must be changed, oral faropenem, cefditoren, or cefcapene or i.v. panipenem or vancomycin are recommended for resistant *S. pneumoniae*, and oral cefditoren or azithromycin or i.v. piperacillin, ceftriaxone, or meropenem are recommended for BLNAR strains. Clindamycin resistance among GAS and *S. aureus* and macrolide-resistant *Mycoplasma* is a problem. Macrolide-resistant *Mycoplasma* was first detected in culture and by PCR in 2000, and many strains are highly resistant to erythromycin.[42,43] Although the period of fever following macrolide administration is significantly longer than that for infections by susceptible strains (mean duration of fever: 4.3 days for resistant strains vs 1.4 days for susceptible strains), the clinical symptoms are not more severe.[43] Changing treatment to a tetracycline antibiotic should be considered if fever persists for more than 48 h after macrolide antibiotic initiation.

The *Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007* are summarized here with a focus on pneumonia. Only selected tables and figures to illustrate the *Guidelines* could be reproduced here due to limited space.

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References

1 Uehara S, Sunakawa K. (Editorial Supervisors). *Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2004*. KYOWA KIKAKU Ltd., Tokyo, 2004 (in Japanese).
2 Uehara S, Sunakawa K. (Editorial Supervisors). *Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*. KYOWA KIKAKU Ltd., Tokyo, 2007 (in Japanese).
3 Uehara S. Microbiological examination, bacterial infection. In: Kobayashi N (ed). *New Encyclopedia of Pediatrics 9A. Pediatric Respiratory Infectious Diseases 1*. Nakayama Shoten, Tokyo, 1982; 240–61 (in Japanese).
4 Moffet HL. *Pediatric Infectious Disease – A Problem Oriented Approach*, 3rd edn. Lippincott, Philadelphia, PA, 1989; 146–96.
5 Silverman M, Stratton D, Diallo A, Egler LJ. Diagnosis of acute bacterial pneumonia in Nigerian children. Value of needle aspiration of lung of countercurrent immunoelectrophoresis. *Arch. Dis. Child* 1977; **52**: 925–31.
6 Uehara S. Fundamental studies on guidelines for diagnosis and treatment of childhood pneumonia 1) Introduction: Nationwide survey of causative organisms in childhood pneumonia by golden standard. *Jpn. J. Pediatr. Pulmonol.* 2003; **14**: 172–77 (in Japanese with English abstract).
7 Kubo S, Funabashi S, Uehara S, Toba T, Muramatsu Y, Sanada K. Clinical aspects of asthmatic bronchitis and chronic bronchitis in infants and children. *J. Asthma Res.* 1978; **15**: 99–132.
8 Uehara S. A method of bacteriological examination of washed sputum in infants and children. *Acta Pediatr. Jpn.* 1988; **30**: 253–60.
9 Bucher U. Die Sputimuntersuchung bei unspezifischen Krankheiten der tiefen Luftwege. Verlag Hans Huber, Bern und Stuttgart, 1965 (in German).
10 Geckler RW, Gremillion DH, McAllister CK, Ellenbogen C. Microscopic and bacteriological comparison of paired sputa and transtracheal aspirates. *J. Clin. Microbiol.* 1977; **6**: 396–9.
11 Takeda N, Kuroki H, Ishikawa N *et al*. The usefulness of washed sputum culture in children with lower respiratory infections. *J. Jpn. Pediatr. Soc.* 1998; **102**: 975–80 (in Japanese with English abstract).
12 Adegbola RA, Obaro SK, Biney E, Greenwood BM. Evaluation of Binax now *Streptococcus pneumoniae* urinary antigen test in children in a community with a high carriage rate of pneumococcus. *Pediatr. Infect. Dis. J.* 2001; **20**: 718–19.
13 Baron EJ. (Coordinating editor). *Cumitech (Cumulative Techniques and Procedures in Clinical Microbiology) 1C: Blood Cultures IV*. ASM Press, Washington, DC, 2005.
14 Jacobs E. Serological diagnosis of *Mycoplasma pneumoniae* infections: A critical review of current procedures. *Clin. Infect. Dis.* 1993; **17** (Suppl. 1): S79–82.
15 Kishimoto T, Matsushima T, Morikawa T, Kawagoe K. Assay of specific anti-*Chlamydia pneumoniae* antibodies by ELISA method. 3. Setting of serological criteria. *J. Jpn. Assoc. Infect. Dis.* 1999; **73**: 457–66 (in Japanese with English abstract).
16 Numazaki Y, Oshima T, Ohmi A *et al*. A microplate method for isolation of viruses from infants and children with acute respiratory infections. *Microbiol. Immunol.* 1987; **31**: 1085–95.
17 American Academy of Pediatrics. Group A streptococcal infections. In: Pickering LK, Baker CJ, Long SS (eds). *Red Book: 2006 Report of the Committee on Infectious Diseases*, 27th edn. American Academy of Pediatrics, Elk Grove Village, IL, 2006; 610–20.
18 Peltola H. *Haemophilus influenzae* type b disease and vaccination in Europe: Lessons learned. *Pediatr. Infect. Dis. J.* 1998; **17**: S126–32.
19 Cherry JD. Acute bronchitis. In: Feigin RD, Cherry JD, Demmler GJ, Kaplan S (eds). *Textbook of Pediatric Infectious Diseases*, 5th edn. W.B. Saunders, Philadelphia, PA, 2004; 266–9.
20 Hall CB, McBride JT. Bronchiolitis. In: Mandell G (ed). *Principles and Practice of Infectious Diseases*. Churchill Livingstone, Philadelphia, 2005; 812–19.
21 Sasaki A, Suzuki H, Saito R *et al*. Prevalence of human metapneumovirus and influenza virus infections among Japanese children during two successive winters. *Pediatr. Infect. Dis. J.* 2005; **24**: 905–8.

22 McIntosh K. Community-acquired pneumonia in children. *N. Engl. J. Med.* 2002; **346**: 429–37.
23 Ouchi K, Kanamoto Y, Ushio M. Prevalence of antibodies to *Chlamydia pneumoniae* (strain TWAR) and other *Chlamydia* in Japan. *J. Jpn. Assoc. Infect. Dis.* 1991; **65**: 19–25 (in Japanese with English abstract).
24 Kishimoto T. Studies on *Chlamydia pneumoniae*, strain TWAR, infection 2. Seroepidemiology of TWAR on healthy controls and patients with acute respiratory infections. *J. Jpn. Assoc. Infect. Dis.* 1990; **64**: 986–93 (in Japanese with English abstract).
25 Grayston JT. *Chlamydia pneumoniae* (TWAR) infections in children. *Pediatr. Infect. Dis. J.* 1994; **13**: 675–84.
26 Ishiwada N, Kurosaki T, Toba T, Niimi H. Etiology of pediatric inpatients with pneumonia-analysis of clinical symptoms, physical examination and simple laboratory findings. *J. Jpn. Assoc. Infect. Dis.* 1995; **69**: 284–90 (in Japanese with English abstract).
27 Kurosaki T. Fundamental studies on guidelines for diagnosis and treatment of childhood pneumonia, management of childhood pneumonia. *Jpn. J Pediatr. Pulmonol.* 2003; **14**: 198–204 (in Japanese with English abstract).
28 Swischuk LE, Hayden CK Jr. Viral vs. bacterial pulmonary infections in children (is roentgenographic differentiation possible?). *Pediatr. Radiol.* 1986; **16**: 278–84.
29 Khamapirad T, Glezen WP. Clinical and radiographic assessment of acute lower respiratory tract disease in infants and children. *Semin. Respir. Infect.* 1987; **2**: 130–44.
30 World Health Organization. Programme for the control of acute respiratory infections: Technical bases for the WHO recommendations on the management of pneumonia in children at first-level health facilities. WHO/ARI/91.20.
31 Heffelfinger JD, Dowell SF, Jorgensen JH *et al.* Management of community-acquired pneumonia in the era of pneumococcal resistance: A report from the Drug-Resistant *Streptococcus pneumoniae* Therapeutic Working Group. *Arch. Intern. Med.* 2000; **160**: 1399–408.
32 Kurosaki T. Bronchopulmonary infections caused by penicillin-resistant *Streptococcus pneumoniae* – Problems in selection of antimicrobials and anticipated vaccine therapy. *Igaku no Ayumi* 2004; **208**: 19–22 (in Japanese).
33 NCCLS. Performance standards for antimicrobial susceptibility testing; eleventh informational supplement. MIC interpretive standards (μg/mL) for *Haemophilus* spp. 2001; **21**: 98–9, in NCCLS document M100-S11.
34 Sudo F, Ishiwada N, Hoshino T *et al.* Clinical effects of piperacillin and tazobactam/piperacillin on *Haemophilus influenzae* lower respiratory tract infection in pediatric patients. *J. Jpn. Assoc. Infect. Dis.* 2005; **79**: 637–43 (in Japanese with English abstract).
35 American Academy of Pediatrics. *Moraxella catarrhalis* infection. In: Abramson JS (ed). *Red Book:2003 Report of the Committee on Infectious Diseases*. American Academy of Pediatrics, Elk Grove Village, IL, 2003; 438–9.
36 Oshima H, Kurosaki T, Ota F, Tamai K, Uehara S, Niimi H. Clinical studies on lower respiratory tract infections caused by *Moraxella catarrhalis* in childhood. *J. Jpn. Pediatr. Soc.* 1998; **102**: 23–8 (in Japanese with English abstract).
37 Ubukata K. Problems associated with high prevalence of multidrug-resistant bacteria in patients with community-acquired infections. *J. Infect. Chemother.* 2003; **9**: 285–91.
38 Sakata H. Evaluation of antibiotic treatment of pneumonia presented in Guideline for Management of Respiratory Infectious Diseases in Children in Japan 2004. *Jpn. J. Pediatr. Pulmonol.* 2006; **17**: 24–30 (in Japanese with English abstract).
39 Fukasawa C, Ishiwada N, Nagai F *et al.* Assessment of clinical usefulness of 'Guidelines for Pediatrics Respiratory Infection 2004'; retrospective analysis of pediatric inpatient with pneumonia. *J. Jpn. Pediatr. Soc.* 2006; **110**: 1256–62 (in Japanese with English abstract).
40 Hasui M, Kobayashi Y, Harada Y *et al.* Clinical and bacteriological studies of ceftriaxone (CTRX) once daily administration in pediatric patients with respiratory tract infections. *Jpn. J. Antibiot.* 2001; **54**: 532–40 (in Japanese).
41 Ubukata K, Chiba N, Hasegawa K, Kobayashi R, Iwata S, Sunakawa K. Antibiotic susceptibility in relation to penicillin-binding protein genes and serotype distribution of *Streptococcus pneumoniae* strains responsible for meningitis in Japan, 1999 to 2002. *Antimicrob. Agents Chemother.* 2004; **48**: 1488–94.
42 Morozumi M, Hasegawa K, Kobayashi R *et al.* Emergence of macrolide-resistant *Mycoplasma pneumoniae* with a 23S rRNA gene mutation. *Antimicrob. Agents Chemother.* 2005; **49**: 2302–6.
43 Suzuki S, Yamazaki T, Narita M *et al.* Clinical evaluation of macrolide-resistant *Mycoplasma pneumoniae*. *Antimicrob. Agents Chemother.* 2006; **50**: 709–12.

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Evaluation of ELISA Kit for Detection of Pertussis-associated IgG Antibodies

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Abstract

We had an opportunity to test a kit manufactured by Denka Seiken Co., Ltd. that measures anti-PT and anti-FHA antibodies (hereinafter referred to as "this product") and evaluate its fundamental performance. This product was an ELISA reagent with a 96-well microplate and we found that it was versatile and easy-to-handle, and had a high reproducibility of data. We also observed a high correlation of measured values between an in vitro diagnostic using a bead-based ELISA test and this product.

Introduction

Although pertussis is a vaccine-preventable disease, it may become severe in some unvaccinated infants. Meanwhile, if adults are infected, their symptoms are not always typical coughs and it is difficult to diagnose. Furthermore, adult pertussis patients are sources of infection in unvaccinated infants.[1-4]

In 1981, Japan lead the world in the introduction of diphtheria pertussis tetanus vaccine (DTaP) including the acellular pertussis vaccine, which mainly consisted of PT (pertussis toxin) and FHA (filamentous hemagglutinin), and the number of reported pertussis patients decreased significantly, but in recent years, the number of adult pertussis patients has relatively increased.[5] It has been considered that they replace DT routine vaccination of period II with DTaP to discourage this tendency.[6] IgG antibodies against PT and FHA must be measured to evaluate the vaccine effect, and these have been used to diagnose pertussis.[7,8] In regards to the measurement of serum antibodies, although the measurement of agglutination titers by bacterial aggregation is more widespread in routine clinical practice in Japan, care should be taken in the interpretation of most agglutinin titers because some of the DTaP vaccines include agglutinin[7]. It has been difficult to compare data of agglutination titers with that of Western countries because the ELISA test is usually used to measure anti-PT antibodies in the West.

In this study, we tested a kit manufactured by Denka Seiken Co., Ltd. that measures anti-PT and anti-FHA antibodies and evaluated its fundamental performance.

I. Materials and Methods

1. Measurement Kit

The reagents used in this study were included in an ELISA kit newly developed by Denka Seiken

Key words : Pertussis kit, pertussis toxin (PT), filamentous hemagglutinin (FHA)

Co., Ltd. that measures serum anti-PT and anti-FHA IgG antibodies. This product consisted of the following reagents：① A PT immobilized plate, a freeze-dried plate, ② A FHA immobilized plate, a freeze-dried plate, ③ Buffer solution, liquid form, ④ Enzyme-labeled antibodies (for PT), liquid form, ⑤ Enzyme-labeled antibodies (for FHA), liquid form, ⑥ Substrate solution, liquid form, ⑦ Concentrated washing solution, liquid form, ⑧ Stop solution, liquid form

2. Measurement Principle and Procedure for this product

The measurement principle for this product is a general ELISA test. The following is the practical procedure provided by Denka Seiken Co., Ltd.

＜Preparation of Reagents＞

Dilute the concentrated washing solution ten times with purified water depending on the number of samples, and use as a washing solution. Other reagents should be used without dilution.

＜Preparation of Pre-diluted Samples＞

Add 2 mL of buffer solution into some small test tubes according to the number of samples. Then distribute 10 μL of each sample into each test tube and mix well. Use these solutions as pre-diluted samples.

＜Procedure＞

Use control for PT or FHA provided separately to prepare a calibration curve. Pull out PT or FHA immobilized plates from aluminum bags according to the number of samples and set in the plate holder. Prepare a well for each blank, pre-diluted sample and each antibody titer (0, 5, 10, 20, 40, 80 and 160 EU/mL) of the control for PT or FHA.

＜Step 1 (The Primary Reaction)＞ Instillations of Pre-diluted Samples and Controls

1) Add 100 μL of corresponding control or pre-diluted sample to PT or FHA immobilized plates in a certain order and at regular intervals. No sample or control should be added to the wells of the blank.

2) Mix for a few seconds with a microplate mixer. Cover with plastic wrap, etc., and make them stand still for 1 hour at 20-30℃.

＜Step 2 (The Secondary Reaction)＞ Instillations of Solutions of Enzyme-labeled Antibodies

1) Remove the reaction solution in each well by aspiration in the same order and at the same intervals as in ＜Step 1＞.

2) Add approximately 200 μL of washing solution to each well and mix for a few seconds with a microplate mixer. Remove the solution again by aspiration. Repeat these washing procedures twice more. Flip the plates upside down and beat them on some clean paper towels to remove the residual washing solution completely.

3) Add 100 μL of solutions of enzyme-labeled antibodies (for PT or for FHA) to each well in the same order and at the same intervals as in ＜Step 1＞ and mix for a few seconds with a microplate mixer. Cover with plastic wrap, etc., and make them stand still for 1 hour at 20-30℃. No solutions of enzyme-labeled antibodies should be added to the wells of the blank.

＜Step 3 (Enzyme Reaction)＞ Instillations of Substrate Solution

1) Remove the reaction solution in each well by aspiration in the same order and at the same intervals as in ＜Step 1＞.

2) Add approximately 200 μL of washing solution to each well and mix for a few seconds with a microplate mixer. Remove the solution again by aspiration. Repeat these washing procedures four more times. Flip the plates upside down and beat them on some clean paper towels to remove the residual washing solution completely.

3) Add 100 μL of substrate solution to each well in the same order and at the same intervals as in ＜Step 1＞ and mix for a few seconds with a microplate mixer. Cover with plastic wrap, etc., and make them stand still for 1 hour at 20-30℃ under protection from light. In addition, add 100 μL of the substrate solution to the wells of the blank.

<Step 4> Instillations of Stop Solution and Measurement

1) Add 100 μL of stop solution to each well in the same order and at the same intervals as in <Step 1>. In addition, add 100 μL of the stop solution to the wells of the blank.

2) Measure with an automatic reader (dominant wavelength : 450 nm, reference wavelengths : 600～700 nm) within 30 minutes using the well of the blank as a control.

<Evaluation of Measurement Results>

1) Subtract the absorbance of the blank from that of each control.

2) Confirm that each value obtained by subtracting the absorbance of the blank from that of 10 EU/mL of antibody titer in each control is within 0.15-0.60.

3) Define the value obtained by subtracting the absorbance of the blank from that of sample as "a."

4) Plot antibody titers (EU) of each control on the horizontal axis and values obtained in 1) on the vertical axis to prepare calibration curves.

5) Based on these calibration curves, calculate the antibody titer corresponding to the absorbance of each sample (a) and display. The obtained values should be rounded down to whole numbers.

3. Serum Sample

We used serum samples from 158 patients who had visited National Hospital Organization Fukuoka National Hospital from 2005 to 2010 and had been suspected of pertussis infection.

4. Standard Substance

We dissolved the standard serum JNIH-10 (PT : 250 EU/vial, FHA : 400 EU/vial) obtained from the National Institute of Infectious Diseases in Japan into 1 mL of purified water and used this as a standard substance.

5. International Standard Serum[9)10)]

We dissolved the international standard serum 06/140 (PT : 335 IU/vial, FHA : 130 IU/vial) obtained from the National Institute for Biological Standards and Control (NIBSC, U.K.) into 1 mL of purified water and used this as an international standard serum.

6. Measurement of Serum Samples, the Standard Substance and the International Standard Serum

We measured a twofold dilution series of the standard substance JNIH-10 in accordance with the above procedure and evaluated the range of absorbance and the effects by dilution. We then measured a dilution series of the international standard serum 06/140 as well and compared it with the results in JNIH-10 to evaluate the correlation between the standard unit in Japan (EU) and the international standard unit (IU).

All the serum samples were evaluated by JNIH-10. Antibody titers (EU/mL) were calculated using calibration curves prepared by controls for PT or FHA provided separately.

7. Evaluation of Changes in Measured Values by Repeated Measurement of the Same Sample

We measured two samples of known anti-PT and FHA antibody titers, a total of four samples, four times and calculated the coefficient of variation (CV, %) to evaluate within-run reproducibility of this product. We used two types of samples of known antibody titers, 20 and 80 EU/mL.

8. Correlativity with the Control Product

We used Reagent Wako for Measurement of Pertussis Bacteria Antibody Titer (Wako Pure Chemical Industries, Ltd.) as a control product and evaluated correlativity with this product. Since the measurement range of this product was 1～160 EU/mL and that of the control product was 1～100 EU/mL, if the antibody titer of the sample showed as out of the range for both or either of them, the sample was excluded. We calculated the slopes of regression lines and correlation coefficients for 137 samples of anti-PT antibody titers and 145 samples of anti-FHA antibody titers.

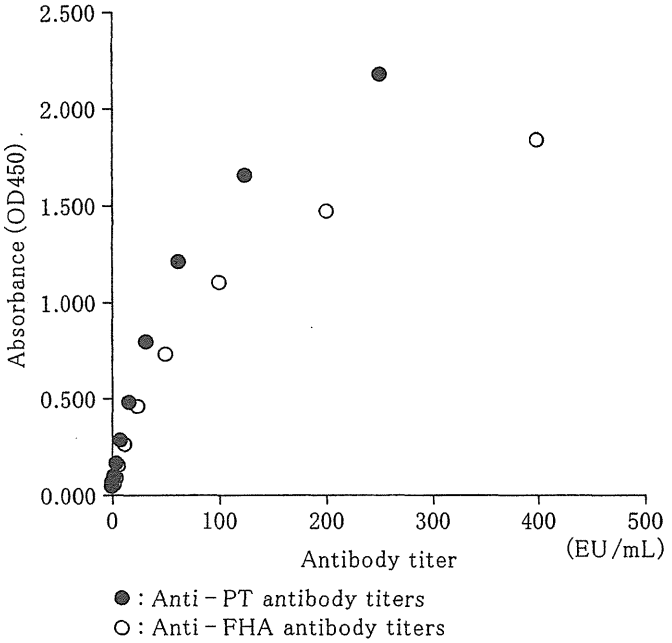

Fig. 1　Changes of absorbance in dilution series of JNIH-10

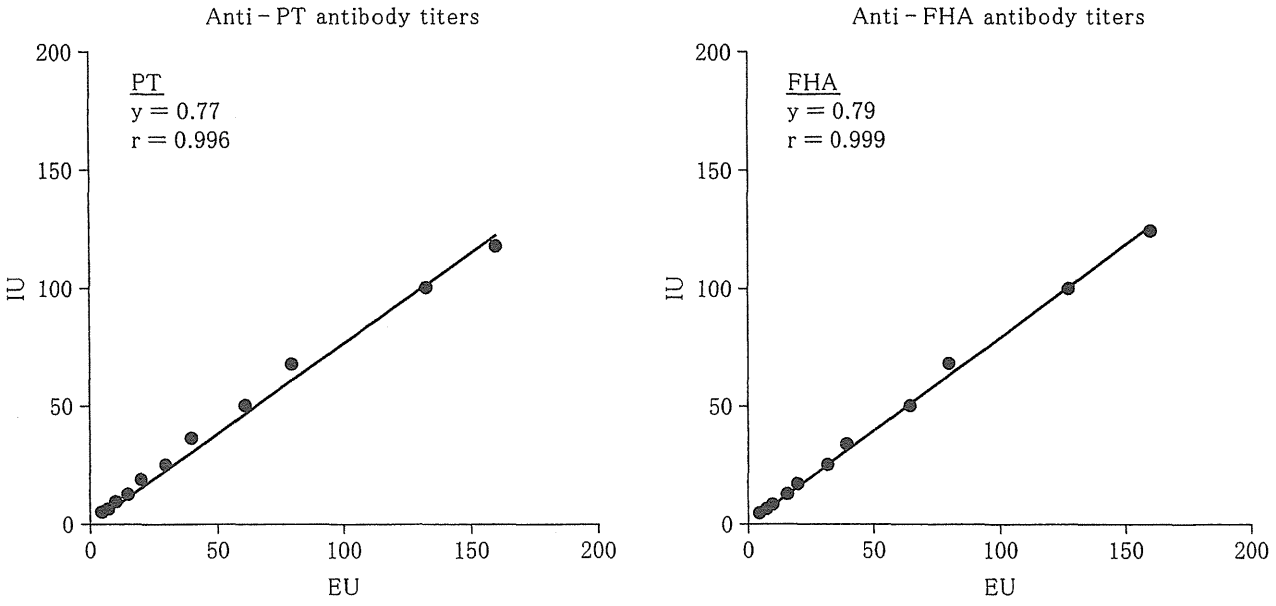

Fig. 2　Comparison between the standard unit in Japan (EU) and the international unit (IU)

II. Results

1. Changes of Absorbance in a Dilution Series of JNIH-10

We prepared a twofold dilution series of JNIH-10 and measured absorbance in accordance with the above procedure (**Fig. 1**). As a result, the absorbance obtained from the pre-diluted sample of the undiluted solution of JNIH-10 (diluted to 201-fold with the buffer solution) showed around 2OD in both anti-PT and FHA antibodies. In the lots of reagents shown in Fig. 1, the absorbance of anti-PT antibodies was usually slightly higher than that of anti-FHA antibodies in the same EU values, but their absorbance was considered to be practically equivalent. It can be considered that increases of absorbance of anti-PT and anti-FHA antibodies slow down as increases of antibody titers and the

Table 1　Simultaneous reproducibility

Samples	Lot# of reagents	CV values (%)		
		First	Second	Third
PT (20 EU/mL)	1	1.3	2.9	2.1
	2	3.6	2.7	4.1
	3	2.8	2.0	5.5
PT (80 EU/mL)	1	4.5	3.9	1.0
	2	2.6	3.9	3.2
	3	2.4	0.5	1.9
FHA (20 EU/mL)	1	2.2	2.1	2.9
	2	2.4	1.1	1.4
	3	2.0	2.7	2.4
FHA (80 EU/mL)	1	2.0	2.4	2.9
	2	0.9	1.9	1.5
	3	2.5	1.2	1.3

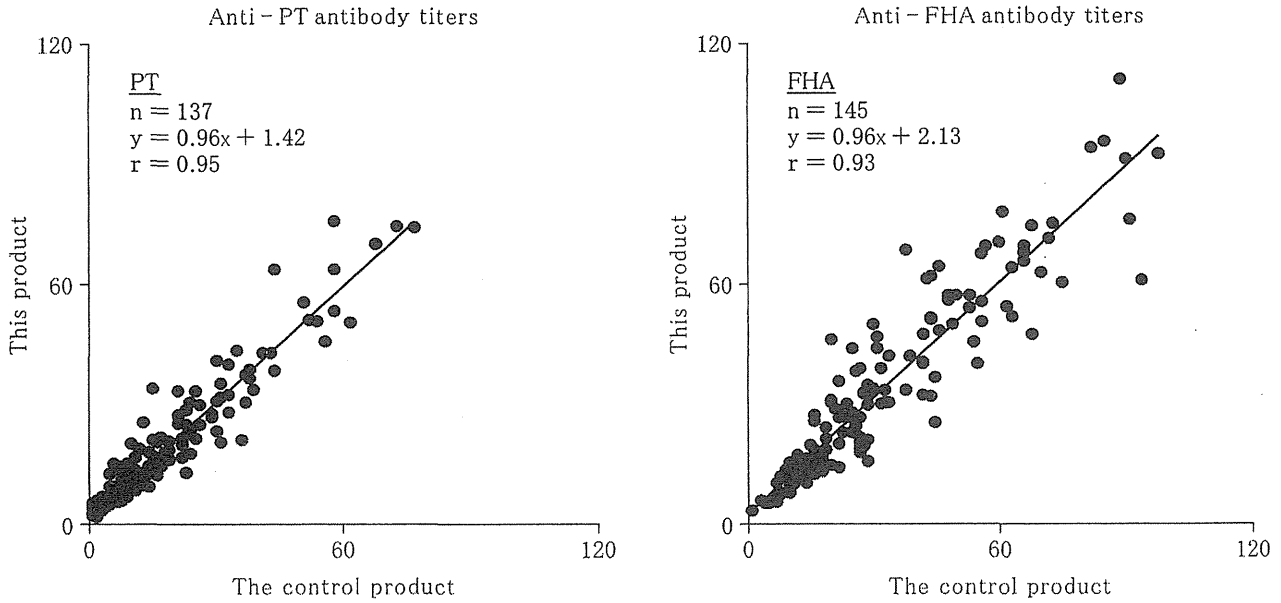

Fig. 3　Correlation with the control product

dilution curves reach plateau. This is a common phenomenon in the plate ELISA test.

2．Comparison between the Standard Unit in Japan (EU) and the International Unit (IU)

We simultaneously measured (seven times per lot) the dilution series of JNIH-10 (**Fig. 2**, x-axis) and the international standard serum 06/140 (Fig. 2, y-axis) using this product to compare them. The results showed a good correlation, y＝0.77x (r＝0.996) in PT and y＝0.79x (r＝0.999) in FHA.

3．Evaluation of Changes in Measurement Values by Repeated Measurement of the Same Sample

As shown in **Table 1**, the coefficient of variation (CV, %) obtained with a four-time measurement of two types of samples with known antibody titers were 0.5～5.5% in anti-PT antibody titers and 0.9～2.9% in anti-FHA antibody titers. These results showed a good reproducibility in all evaluated lots of reagents.

4. Correlation with the Control Product

As shown in **Fig. 3**, the linear regression formula of PT (n=137) was y=0.96x+1.42 (r=0.95) and that of FHA (n=145) was y=0.96x+2.13 (r=0.93), and both of them showed high correlations.

III. Discussion

Since this product adopts a general 96-well microplate, it is considered to have good versatility and can easily conform to automation in sites such as testing centers, which treat many samples. From the results of fundamental performance in this study, this product is a stable reagent with a good reproducibility and is expected to enable the comparison of data with that by Reagent Wako ELISA test due to a high correlation with that test, which is currently the only reagent used to measure anti-PT and anti-FHA antibodies in Japan. Furthermore, this product is also considered to allow the possibility of comparing data with anti-PT antibody titers obtained in IU in other countries[11)12)]. We believe that this product can be widely used in support of clinical diagnosis of pertussis, as well as in the evaluation of vaccine effects, epidemiological studies and for other purposes.

However, the results of this study were insufficient to calculate a conversion factor between EU and IU. The purities of antigens, purification methods, strains, compositions of buffer solutions, etc., used in the test differ for every manufacturer of reagents, which affects the reactivity of reference and standard substances. Since serum antibodies are polyclonal, the composition rates of recognition epitopes may differ in every serum. In view of the fact that a certain tendency is expected to be present in the products by the same manufacturer, we estimated that the IU value was approximately 70-80% of the EU value in this product.

References

1) Cherry JD：The role of Bordetella pertussis infections in adults in the epidemiology of pertussis. Dev Biol Stand **89**：181-186, 1997.
2) Deen JL, Mink CA, Cherry JD et al：Household contact study of Bordetella pertussis infections. Clin Infect Dis **21**(5)：1211-1219, 1995.
3) Long SS, Welkon CJ, Clark JL：Widespread silent transmission of pertussis in families：antibody correlates of infection and symptomatology. J Infect Dis **161**(3)：480-486, 1990.
4) Nelson JD：The changing epidemiology of pertussis in young infants. The role of adults as reservoirs of infection. Am J Dis Child **132**(4)：371-373, 1978.
5) Infectious Agents Surveillance Report (monthly report) (IASR) Special topic：Pertussis 29 (3), the National Institute of Infectious Diseases, the Tuberculosis and Infectious Diseases Control Division in the Health Policy Bureau in the Ministry of Health, Labour and Welfare, 2008 [Japanese]
6) Okada K et al：Safe and effective booster immunization using DTaP in teenagers. Vaccine **28**(48)：7626-7633, 2010.
7) Tatsuo Kato：DPT. The Journal of Pediatric Practice **10**(49) 2275-2281, 1990. [Japanese]
8) Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007, the Committee of Preparation of Guidelines for the Management of Respiratory Infectious Diseases in Children, Kyowa Kikaku Ltd., pp. 112-117, 2007. [Japanese]
9) Xing D, Wirsing von Konig CH, Newland P et al：Characterization of reference materials for human antiserum to pertussis antigens by an international collaborative study. Clin Vaccine Immunol **16**(3)：303-311, 2009.
10) Dalby T, Sorensen C, Petersen JW et al：Pertussis serology：assessment of IgG anti-PT ELISA for replacement of the CHO cell assay. APMIS **118**(12)：968-972, 2010.
11) Guiso N. Berbers G. Fry Nk et al.：What to do and what not to do in serological diagnosis of pertussis：recommendations from EU reference laboratories. Eur J Clin Microbiol Infect Dis **30**(3)：307-312, 2011.
12) Riffelmann M, Thiel K, Schmetz J et al：Performance of commercial enzyme-linked immunosorbent assays for detection of antibodies to Bordetella Pertussis. J Clin Microbiol **48**(12)：4459-4463, 2010.

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Diminished immunogenicity to pandemic H1N1 2009 influenza vaccine in subjects with severe motor and intellectual disability

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Influenza vaccine

ABSTRACT

Subjects with severe motor and intellectual disability (SMID) are considered to be debilitated and at high risk of influenza infection. However, the safety and immunogenicity of pandemic H1N1 (pH1N1) vaccine in these subjects have not been reported. We measured the hemagglutination inhibition antibody titer and calculated the geometric mean titer ratio (GMTR), seroprotection rate, and seroconversion rate in 104 subjects with SMID (mean age ± standard deviation 40.1 ± 12.9 years), and in 179 healthcare workers (40.7 ± 10.4 years) in a long-term care facility. Antibody responses after the first dose of pH1N1 vaccine among workers were greater than the European Medicines Evaluation Agency criteria and US Food and Drug Administration (FDA) criteria: the seroprotection rate was 79.9% (95% confidence interval (CI) 73.3–85.5), the seroconversion rate was 77.9% (95%CI: 70.8–84.0), and GMTR was 7.3 (95%CI: 6.9–7.8). Responses among subjects with SMID were lower than the FDA criteria: the seroprotection rate was 56.3% (95%CI: 46.2–66.1), the seroconversion rate was 54.1% (95%CI: 43.7–64.2), and GMTR was 5.4 (95%CI: 4.9–5.9). Any additional antibody response induced by the second dose of vaccine among subjects with SMID was limited. Multivariate analysis indicated that subjects with SMID had a significantly lower seroprotection rate (odds ratio (OR) 0.37, 95%CI: 0.20–0.66) and seroconversion rate (OR 0.34, 95%CI: 0.20–0.59) than healthcare workers. No serious adverse reaction was reported in either group. These results indicate that a single dose of pH1N1 vaccine does not induce sufficient immunity among subjects with SMID, and a second dose is likely to be ineffective because of diminished immunogenicity. Further study is required to determine if vaccination over consecutive influenza seasons can improve immunogenicity in subjects with SMID.

1. Introduction

In June 2009, the first influenza pandemic of the 21st century, originating from swine influenza A (H1N1) 2009 virus, was declared [1]. After the pandemic, underlying medical conditions, such as chronic respiratory diseases, chronic cardiovascular diseases, chronic renal disease, chronic liver diseases, neurologic disease, hematologic disease, diabetes, and immunosuppression caused by therapy or illness, were reported to be associated with a higher risk of hospitalization of patients with the 2009 pandemic influenza A/H1N1 (pH1N1) virus infection [2–4], as well as severe illness associated with seasonal influenza [5,6]. Influenza virus vaccination is the primary method to prevent influenza and its severe complications, thus information on safety and immunogenicity of influenza vaccination in high-risk groups, including those with underlying medical conditions, is needed. Recent studies have confirmed the safety and immunogenicity of pH1N1 vaccine in healthy individuals [7], but studies in high-risk groups were limited [8–10].

Severe motor and intellectual disability (SMID) is defined as being bedridden or only able to sit, crawl, or walk with support, and having a low intelligence quotient (<35); 40–60% of SMID subjects reside in chronic-care facilities in Japan [11]. Their lifespan is lower than in the general population, and the major cause of death was reported to be respiratory disease [11]. The main causes of disability were hypoxic encephalopathy/postnatal distress, unknown

Abbreviations: SMID, severe motor and intellectual disability; EMEA, European Medicines Evaluation Agency; FDA, Food and Drug Administration; GMTR, geometric mean titer ratio; HAI, hemagglutination-inhibition.

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prenatal factors, external factors including brain injury, meningitis/encephalitis, hydrocephalus and epilepsy [11], and subjects are considered to be debilitated or immunocompromised [12]. Their immunogenicity to trivalent influenza vaccination (A/H1N1, A/H3N2, and type B influenza) has been reported to be lower than in healthy individuals, a booster effect was absent, and age had a greater influence on immunogenicity than the severity of symptoms [12,13]. Thus immunogenicity to pH1N1 vaccine is suspected to be diminished among subjects with SMID, although no information has been reported yet.

In this report, we compare the safety and immunogenicity profile of unadjuvanted pH1N1 vaccine between subjects with SMID and healthy individuals. In addition, we evaluate the immunogenicity according to age, pre-vaccination serostatus, underlying medical conditions and severity of symptoms, and the effect of a second dose of vaccine on immunogenicity.

2. Materials and methods

2.1. Study design

This prospective controlled cohort study among subjects with SMID and healthcare workers was conducted at along-term care facility in Hokkaido Prefecture located in northern Japan between October 2009 and May 2010. Of 110 residents with SMID, 104 subjects, whose guardians gave written informed consent for their participation in this study, were included in the study. They mainly suffered from cerebral palsy, epilepsy, and intellectual disorders. Of 185 healthcare workers employed at the nursing home, 179 gave written informed consent for participation in the study as healthy controls. None had a history of allergy to eggs, past anaphylaxis to vaccine components or either respiratory illness or fever related to suspected 2009 H1N1 infection at the time of vaccination. No participant had received the 2009/2010 seasonal influenza vaccine prior to A/H1N1 vaccine.

A single dose of 0.5 mL monovalent inactivated unadjuvanted split-virus 2009 pH1N1 vaccine contained at least 15 μg of hemagglutinin antigen to A/California/7/2009 (A/H1N1)v-like strain. Subjects with SMID were administered two doses subcutaneously, 3 weeks apart. The vaccine was produced by the Research Foundation for Microbial Disease of Osaka University, Osaka (lot no. HP01A in 2009). Healthcare workers were administered one dose subcutaneously. Among them, 127 received the same vaccine as the SMID group and 52 received a vaccine from the Chemo-Sero-Therapeutic Research Institute (lot no. SL02B in 2009).

All vaccinated subjects were carefully observed for anaphylactic shock for at least 30 min after vaccination, and for adverse reactions for 48 h following vaccination, either local (erythema, swelling, induration, itching, and pain) or general (fever, fatigue, myalgia or arthralgia, headache, and rash). Regarding subjects with SMID, healthcare workers monitored and recorded their local and general reactions based on a unified questionnaire. In addition, their body temperature, amount of food intake in each meal, frequency of urination and defecation, and irritability as routine health-checks were monitored and recorded by healthcare workers and we collected the information retrospectively. The healthcare workers self-assessed their local and general reactions based on a unified questionnaire.

Serum samples were collected just prior to the first dose (S0), 3 weeks after the first dose (S1), 4 weeks after the second dose (S2) (only for subjects with SMID), and 6 months after the first dose (S3). All serum specimens were stored at −40 °C until assayed. During the S0 and S3 periods, we followed all study participants prospectively to monitor influenza infection and later adverse events. When the participants suffered any influenza-like symptoms, such as sudden fever (temperature ≥37.8 °C) and respiratory or general symptoms, throat swabs were collected and tested using the rapid diagnosis kit for influenza, which utilizes an immunochromatographic method. To confirm the existence and strain of the influenza virus, throat swabs from kit-diagnosed patients would be sent to our laboratory.

The study protocol was approved by the Institutional Review Board of the Saga University Faculty of Medicine, and conducted in accordance with the principles of the Declaration of Helsinki and Japanese regulatory requirements.

2.2. Measurement of immunogenicity to vaccination

The serum antibody titer against the vaccine strain was measured by the hemagglutination-inhibition (HAI) assay according to standard methods using chicken erythrocytes [14,15]. Serum samples were treated with receptor destroying enzyme (Vibrio cholera filtrate, Denka Seiken, Tokyo, Japan) to inactivate non-specific inhibitors. All samples were assayed at the same time at the laboratory of the Research Foundation for Microbial Disease of Osaka University.

The following variables were calculated: geometric mean titer (GMT) for HAI, the seroprotection rate (the proportion of vaccinations with serum HAI titer ≥ 1:40), seroconversion rate (the proportion of subjects with a pre-vaccination HAI antibody titer < 1:10 and post-vaccination titer HAI ≥ 1:40, or a pre-vaccination titer ≥ 1:10 and a 4-fold antibody increase after vaccination). In these calculations, if HAI titers were below the detection limit (i.e., <1:10 or >1:5120), then we assigned them as 1:5 or 1:5120. The results of GMT at S1, S2, S3 were compared with the result at S0, then the GMT ratio (GMTR) was calculated.

According to the European Medicines Evaluation Agency (EMEA) criteria for evaluating HAI antibody responses to seasonal vaccine [16], a seroprotection rate > 70% or a seroconversion rate >40% or a mean geometric increase > 2.5 are considered the cut-off values for vaccine immunogenicity in adults 18–60 years of age. The US FDA define an adequate response criteria as follows [17]: the lower limit of the two-sided 95% confidence interval (CI) for the seroprotection rate was ≥70% or that for seroconversion rate was ≥40%.

2.3. Statistical analysis

The chi-square test or Fisher's exact test was used to compare the baseline characteristics or rates of seroprotection and seroconversion between SMID subjects and healthcare workers. The 95%CI of rates of seroprotection and seroconversion were calculated with the exact binomial distribution for proportions. The Wilcoxon rank sum test was used to compare GMT and GMTR between the two groups, while the Wilcoxon signed-rank test was used to determine the significance of the increase in titers after vaccination in each group. To compare the immune response to pH1N1 vaccination between subjects with SMID and healthcare workers with consideration of influenza of baseline characteristics and serostatus, we combined data of these two groups. Univariate and multivariate logistic regression modeling were used to obtain crude and adjusted odds ratio (OR) and 95%CI of the association of baseline characteristics and serostatus with immunogenicity. Hypothesis testing was conducted with the use of two-sided tests, with an α of 0.05 considered to indicate statistical significance. All statistical analyses were performed using the SAS software (version 9.1).

3. Results

Characteristics of the 104 subjects with SMID (57 males, 47 females; mean age ± standard deviation 40.1 ± 12.9 years) and 179 healthcare workers (58 males, 121 females; mean age 40.7 ± 10.4

Table 1
Characteristics of the study subjects.

	Subjects with SMID (n = 104)		Healthcare workers (n = 179)	
	n	%	n	%
Age in years (%)				
20–29	25	24.0	27	15.1
30–39	26	25.0	65	36.3
40–49	25	24.0	44	24.6
50–59	28	26.9	43	24.0
Median of age (years)	40		39	
Female (%)	47	45.2	121	67.6[a]
Chronic conditions (%)				
Asthma	10	9.6	2	1.1[a]
Chronic heart failure	1	1.0	0	0.0
Cerebrovascular disease	1	1.0	0	0.0
Liver dysfunction	6	5.8	0	0.0[a]
Diabetes	1	1.0	0	0.0
Atopy	0	0.0	5	2.8
Other	5	4.8	5	2.8
Pre-vaccination HAI antibody titer				
<1:10	74	71.2	87	48.6[a]
1:10–1:20	25	24.0	76	42.5
≥1:40	5	4.8	16	8.9
Seasonal influenza vaccine (2008/2009 season) in the prior 12 months				
Yes	104	100.0	177	98.9

SMID: severe motor and intellectual disabilities; HAI: hemagglutination-inhibition.
[a] p value < 0.05 for comparison between groups with the chi-square test or Fisher's exact test.

years) are shown in Table 1. The age distribution was not different between the two groups and the proportion of females was smaller in the SMID group, which also had a greater proportion of subjects with asthma and liver dysfunction. The distribution of pre-vaccination HAI antibody was significantly different between the two groups: HAI antibodies were detected (titer ≥ 1:10) in 30 people with SMID (28.8%) and in 92 healthcare workers (51.4%). The proportion of seronegative subjects was significantly greater in subjects with SMID (71.2%) than in healthcare workers (48.6%). All subjects with SMID and 177 healthcare workers (98.9%) had received seasonal influenza vaccine (2008/2009 season) in the previous 12 months.

During the follow-up period, none was confirmed to have influenza virus infection by rapid kit in two groups, whereas one patient with SMID showed a 4-fold HAI increase at 6 months after the first dose of the vaccine. The data of this person were not used for persistence assessment of antibody at 6 months after vaccination, because of an apparent infection.

3.1. Immunogenicity

After the first dose of the vaccine, both groups showed a significant increase in antibody titer and seroprotection rate, although the immune response was significantly lower in subjects with SMID compared with healthcare workers (Table 2 and Fig. 1A and B). In healthcare workers, the seroprotection rate (79.9%, 95%CI: 73.3–85.5), seroconversion rate (77.9%, 95%CI: 70.8–84.0), and GMTR (7.3, 95%CI: 6.9–7.8) were greater than the EMEA and FDA criteria. On the other hand, in subjects with SMID, the seroprotection rate (56.3%, 95%CI: 46.2–66.1), seroconversion rate (54.1%, 95%CI: 43.7–64.2), and GMTR (5.4, 95%CI: 4.9–5.9) were greater than the EMEA criteria, but were lower than the FDA criteria. An additional antibody response induced by the second dose of vaccine was minimal in SMID subjects, and was not enough to reach the FDA criteria. Both GMT and seroprotection rate were lower than those in healthcare workers after the first dose (Table 2). The GMT and seroprotection rate at 6 months after vaccination were almost half the level at 3 weeks, but they were still significantly higher than the pre-vaccination level in both groups (Table 2 and Fig. 1A and B).

The pre-vaccination HAI antibodies against the vaccine strain had a significant influence on the seroprotection rate after the first vaccination in both groups. A lower seroprotection rate was induced in the subjects seronegative at baseline than in seropositive subjects (Fig. 1C and D). The seroprotection rates after the first vaccination were similar in all age groups of healthcare workers, whereas an age-related difference was observed among subjects with SMID. The seroprotection rates in older age groups (≥40 years old) were lower than those in younger age groups (<40 years old) of SMID subjects (Fig. 1E and F).

We performed additional analysis to examine the effect of baseline characteristics and serostatus on the immune response to pH1N1 vaccination using the combined data of SMID subjects and healthcare workers (Table 3). Subjects with SMID showed a significantly lower seroprotection rate (OR 0.32, 95%CI: 0.19–0.54) and seroconversion rate (OR 0.33, 95%CI: 0.20–0.55) than healthcare workers. Subjects with seropositive antibodies against the vaccine strain before vaccination showed a significantly higher seroprotection rate (OR 4.60, 95%CI: 2.50–8.49) and seroconversion rate (OR 1.73, 95%CI: 1.05–2.85) than subjects who were seronegative before vaccination. A sex-related difference was absent. The oldest age groups (≥50 years old) had a significantly lower seroprotection rate (OR 0.37, 95%CI: 0.16–0.83) than the youngest age groups (<30 years old). Chronic disease did not influence immunogenicity.

After adjusting for age and pre-vaccination HAI serostatus, subjects with SMID had a significantly lower seroprotection rate (OR 0.37, 95%CI: 0.20–0.66) and seroconversion rate (OR 0.34, 95%CI: 0.20–0.59) than healthcare workers (Table 4).

3.2. Safety

No serious adverse events occurred. A few side effects occurred, but all were mild and transient. Among subjects with SMID, two (1.9%) had conjunctiva redness within 24 h after first vaccination, 10 (9.6%) had a temperature 37.0–37.4 °C and three (2.9%) had erythema smaller than 2 cm within 48 h after the first vaccination. After the second vaccination, 11 (10.5%) had a temperature over

Table 2
Immune response among subjects with SMID and healthcare workers.

	Subjects with SMID		Healthcare workers	
	(*n* = 104)	95%CI	(*n* = 179)	95%CI
GMT				
Pre-vaccination	7.2	(6.3–8.3)[a]	9.2	(8.3–10.3)
3 weeks after 1st vaccination	38.9	(30.7–49.4)[a,b]	67.7	(57.1–80.4)[b]
4 weeks after 2nd vaccination	40.8	(33.3–50.0)[b]	–	–
6 month after 1st vaccination	21.8	(17.8–26.8)[b]	26.5	(22.6–31.1)[b]
GMTR				
3 weeks after 1st vaccination	5.4	(4.9–5.9)[a]	7.3	(6.9–7.8)
4 weeks after 2nd vaccination	5.6	(5.3–6.0)	–	–
6 month after 1st vaccination	3.0	(2.8–3.2)	2.9	(2.7–3.0)
Seroprotection rate (%)				
Pre-vaccination	4.8	(1.6–11.0)[c]	8.9	(5.2–14.1)
3 weeks after 1st vaccination	56.3	(46.2–66.1)[c]	79.9	(73.3–85.5)
4 weeks after 2nd vaccination	58.3	(48.1–67.9)	–	–
6 month after 1st vaccination	33.0	(24.1–43.0)[c]	48.0	(40.5–55.6)
Seroconversion rate (%)				
3 weeks after 1st vaccination	54.1	(43.7–64.2)[c]	77.9	(70.8–84.0)
4 weeks after 2nd vaccination	56.1	(45.7–66.1)	–	–

SMID: Severe motor and intellectual disabilities; GMT: Geometric mean titer; GMTR; GMT ratio; CI: confidence interval.
[a] *p* value < 0.05 compared with healthcare workers by Wilcoxon's rank sum test.
[b] *p* value < 0.05 compared with pre-vaccination by Wilcoxon's signed-rank test.
[c] *p* value < 0.05 compared with healthcare workers by chi-square test.

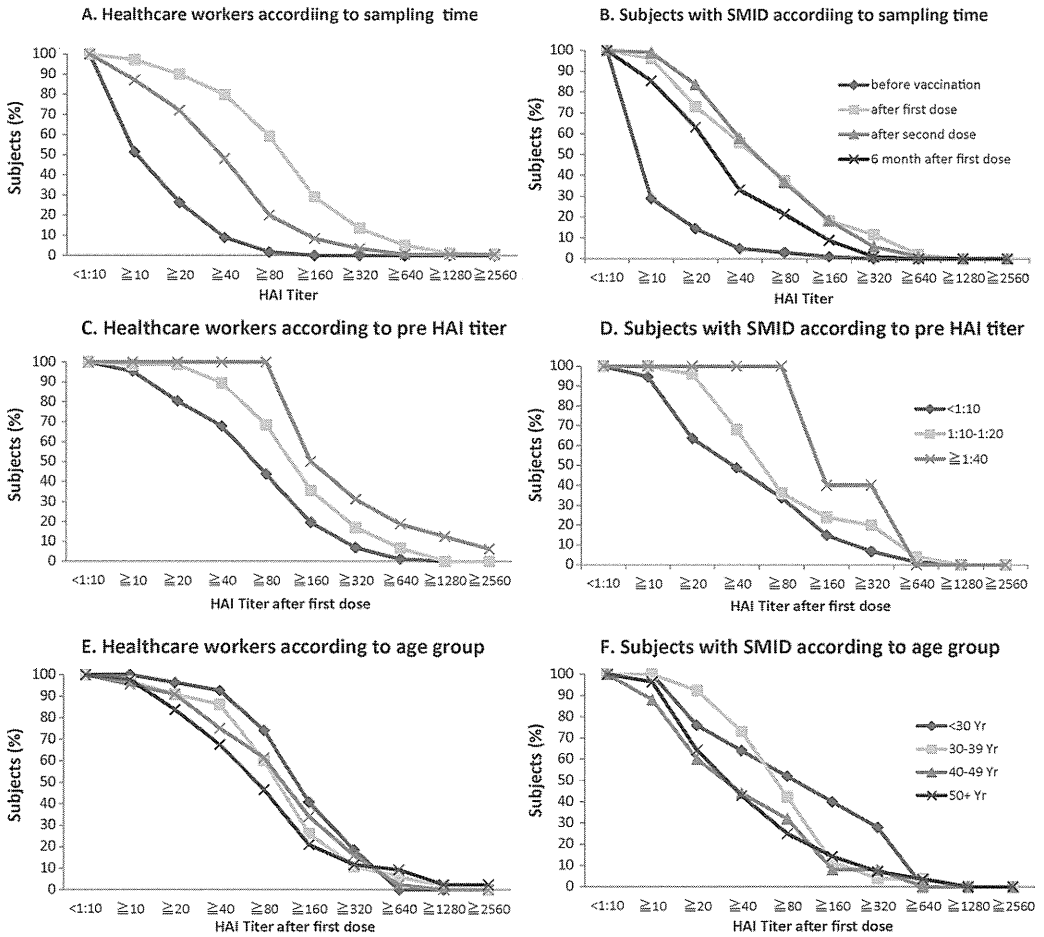

Fig. 1. Reverse cumulative distribution curves of antibody titers in serum according to sampling time, pre-vaccination HAI titer, and age in healthcare workers and subjects with SMID. The percentages of subjects with antibody titer against pandemic H1N1 virus in the HAI assay are shown according to sampling time (Panel A, healthcare workers; Panel B, subjects with SMID), pre-vaccination HAI titer (Panel C, healthcare workers; Panel D, subjects with SMID), and baseline age (Panel E, healthcare workers; Panel F, subjects with SMID).

Table 3
Univariate analysis for evaluation of factors which were associated with immunogenicity after the first vaccination.

		Seroprotection rate				Seroconversion rate			
	All	*n*	(%)	OR	(95%CI)	*n*	(%)	OR	95%CI
Subjects									
Healthcare workers	179	143	79.9	1	Reference	132	73.7	1	Reference
Subjects with SMID	104	58	55.8	0.32	(0.19–0.54)	50	48.1	0.33	(0.20–0.55)
Pre-vaccination HAI antibody titer									
<1:10	161	95	59	1	Reference	95	59	1	Reference
1:10–1:20	101	85	84.2	4.6	(2.50–8.49)	78	77.2	1.73	(1.05–2.85)
≥1:40	21	21	100			9	42.9		
Gender									
Male	115	79	68.7	1	Reference	71	61.7	1	Reference
Female	168	122	72.6	1.21	(0.72–2.03)	111	66.1	1.21	(0.74–1.98)
Age category									
20–29	52	41	78.9	1	Reference	37	71.2	1	Reference
30–39	91	75	82.4	1.26	(0.53–2.96)	65	71.4	1.01	(0.48–2.15)
40–49	69	44	63.8	0.47	(0.21–1.08)	41	59.4	0.59	(0.28–1.28)
50–59	71	41	57.8	0.37	(0.16–0.83)	39	54.9	0.49	(0.23–1.06)
Chronic disease condition									
Asthma (with vs. without)	12	7	58.3	0.56	(0.17–1.80)	7	63.6	0.54	(0.17–1.72)
Liver disfunction (with vs. without)	6	4	66.7	0.82	(0.15–4.52)	4	66.7	1.11	(0.20–6.18)

Table 4
Multivariate analysis for evaluation of factors which were associated with immunogenicity after the first vaccination.

	Seroprotection		Seroconversion	
	OR	95%CI	OR	95%CI
Subjects				
Healthcare workers	1	Reference	1	Reference
Subjects with SMID	0.37	(0.20–0.66)	0.34	(0.20–0.59)
Pre-vaccination HAI antibody titer				
<1:10	1	Reference	1	Reference
≥1:10	4.78	(2.47–9.25)	1.48	(0.87–2.53)
Age category				
20–29	1	Reference	1	Reference
30–39	0.92	(0.37–2.31)	0.78	(0.36–1.73)
40–49	0.27	(0.11–0.68)	0.46	(0.21–1.04)
50–59	0.26	(0.11–0.64)	0.41	(0.19–0.92)

SMID: Severe motor and intellectual disabilities; OR: odds ratio; CI: confidence interval.
ORs were adjusted for age and pre-vaccination HAI serostatus.

37.0 °C (6 subjects had temperature 37.0–37.4 °C, 3 subjects had 37.5–38.0 °C, and 2 subjects had 38.0–38.4 °C), nine (8.7%) had erythema, one (1.0%) had swelling, and one (1.0%) had induration. Among healthcare workers, four (2.2%) had conjunctiva redness within 24 h after the first vaccination, three (1.7%) had a temperature 37.0–37.4 °C, 25 (14.0%) had fatigue, 15 (8.4%) had myalgia or arthralgia, 18 (10.1%) had headache, 15 (8.4%) had erythema, 19 (10.6%) had swelling, nine (5.0%) had induration, 14 (7.8%) had itching, and 20 (11.2%) had pain within 48 h after the first vaccination. All the above side effects were tolerable, and no participant required treatment for them.

4. Discussion

To the best of our knowledge, this is the first report on the immunogenicity and safety of pH1N1 vaccine in subjects with SMID. In this study, a single 15 μg dose of unadjuvanted pH1N1 vaccine induced HAI antibodies in subjects with SMID, but the immunogenicity was lower than that observed in healthcare workers. The seroprotection rate and seroconversion rate among healthcare workers were more than both the EMEA and FDA criteria, whereas those rates in subjects with SMID did not. The seroconversion rate among subjects with SMID (54.1%, 95%CI: 43.7–64.2) met both the EMEA and FDA criteria [16,17]; however, the seroconversion rate among subjects who were seronegative at baseline did not met FDA criteria. In this study, 74 subjects with SMID were seronegative before vaccination, and 36 subjects of these had a response of HAI ≥ 1:40 (seroconversion rate 49%, 95%CI: 37.0–60.0). The seroprotection rate in subjects with SMID did not meet both the EMEA and FDA criteria. In addition, additional antibody response induced by the second dose of vaccine among subjects with SMID was limited. These results indicate that a single dose of pH1N1 vaccine is not enough to induce sufficient immunity among subjects with SMID, and second dose is ineffective, because their immunogenicity is diminished.

The pre-vaccination serostatus was associated with immunogenicity among subjects with SMID, as well as healthcare workers. Subjects who had HAI antibodies against pH1N1 vaccine strain before vaccination showed a greater increase in HAI antibody titer after vaccination by a booster effect. It is reported that priming by prior exposure to related influenza strains through infection or immunization permits a rapid, potent antibody response to immunization [18]. The explanation for existing HAI antibodies to pH1N1 vaccine strain before vaccination are previous influenza infections or cross-reactive antibodies [19]. In this study, the seroprotection rate at baseline among subjects with SMID was significantly lower (5%) than that among healthcare workers (9%). The seroprotection rates were lower than those reported in Australia (30–33%) [19], the USA (20–31%) [20], and Germany (12.5–13.1%) [21], while they were similar to those reported in the UK (2–14%) [22] and China (4.8%) [23] where there were low-level epidemics of pandemic influenza. It is undeniable that there was some degree of previous pH1N1 infection in the study population, despite stringent exclusion criteria [19]. However, subjects with SMID are likely

to have a lower chance of infection withpH1N1 virus because they reside in chronic-care facilities. Kung et al. mentioned in their report that a low prevalence of HAI antibody against pH1N1 virus among study subjects was more likely to give a true primary immune response to vaccine, rather than be complicated by a booster effect of the vaccine [23]. The low seroprotection rate before vaccination in this study might be an advantage. Regarding cross-reactive antibodies, immunotypic similarity between the 2009 H1N1 virus and recent seasonal strains is suspected [19,20]. In our study, both groups had been vaccinated regularly with seasonal vaccine for 5 years or more. The exact reason for the presence of pre-vaccination HAI antibody is unknown, but pre-vaccination antibodies have a benefit on immunogenicity. In this study, the seroprotection rate at 6 months after vaccination declined, but it remained significantly higher than pre-vaccination. Thus, further study to evaluate whether vaccination over consecutive influenza seasons can improve immunogenicity among subjects with SMID is also a matter of great interest.

As in studies of seasonal influenza vaccine [12,13], age was an important factor associated with the level of induced immunity among subjects with SMID, while such an association was absent among healthcare workers. It is supposed that SMID is associated with a rapid aging process [12]. The present result is plausible, because the HAI antibody titer induced by unadjuvantedpH1N1 vaccine in the elderly has been reported to be lower than in healthy adults [24].

Logistic regression modeling showed that subjects with SMID had significantly diminished immunogenicity even after adjustment for age and pre-vaccination serostatus. The OR of SMID status for seroconversion was 0.33, which was better than that reported for hemodialyzed status (OR 0.13) [10]. The seroprotection and seroconversion rates after a single dose of pH1N1 vaccine among subjects with SMID (50% and 45%, respectively) were both higher than those reported in adults with solid tumors and hematological malignancies on active systemic treatment (27% and 19%, respectively) [8]. On the other hand, they were lower than those reported in systemic lupus erythematosus patients, whose seroprotection and seroconversion rates were both 70–80% [9]. It is difficult to compare immunogenicity with other reports because of the existence of pre-vaccination HAI antibodies, which influence the antibody response. However, subjects with SMID can be regarded as an immunosuppressed population. The mechanism of the impaired immune response remains unknown, but we suspected that decreased immunoglobulin levels and abnormalities in T- and B-cells were related, because the subjects with SMID were likely to have a precocious aging process. It has been reported that intrinsic changes in B cells and T cells with age contribute to reduce antibody responses [25–27]. Nutritional status may also play a role, and an association between nutritional status and lower antibody response among the elderly has been reported [28,29]. Subsequent research is needed to further evaluate factors influencing the vaccine response.

The underlining mechanisms of the limited response to the second dose of vaccine in subjects with SMID are still unclear. On the booster vaccination, preexisting memory T cells and memory B cells are capable of mounting a fast and strong response [27], so we speculate that decreased immunological memory contributed to suppress the booster response in subjects with SMID.

Profiles of the adverse events following vaccination, particularly the frequency and severity of local and systemic adverse events, were consistent with previous experience in the seasonal influenza vaccine in subjects with SMID [13] or pH1N1 vaccine in healthy adults [19]. The second dose increased local reactions in subjects with SMID, and this is in agreement with previous reports in healthy adults [24]. Both groups were monitored for 6 months after vaccination, and no vaccine-related serious adverse events were reported. These results indicate unadjuvanted pH1N1 vaccine is safe for subjects with SMID.

Our study has some limitations. The nutritional status may have contributed to diminished immunogenicity among subjects with SMID, but we have no available nutritional data from just before vaccination. The sample size of the present study had a statistical power of at least 80% to detect a seroconversion rate more than 40% in each group; however, it was small for an analysis of individual risk factors, such as chronic disease, severity of SMID, and underlying disease, the most frequent of which were epilepsy and cerebral palsy in this population. There were 31 subjects with epilepsy, 31 subjects with cerebral palsy, and 18 subjects with both, and the GMT after one vaccination was 52.3, 38.3, and 31.7, respectively. These values were statistically lower than that among healthcare workers, but there have been no reports on immunogenicity for these diseases. Down syndrome is the most common chromosomal abnormality, and has been associated with an increased risk for influenza-related complications and a diminished immune response to vaccination [30]. Only six subjects with Down syndrome were involved in the present study, so we calculated their GMT after one dose of vaccine, and found it was quite low (22.4, 95%CI: 7.7–65.5). It is unclear whether this was related to anatomic variations, genetic disorders, an intrinsic immune defect, or other factors such as nutritional status [31]. However, these results suggest that the underlying disease of subjects with SMID may have some influence on their immunogenicity, but it was difficult to evaluate this with adjustment for age and pre-vaccination serostatus because of the small sample in this study. Studies of larger populations with underlying disease are needed to obtain full data on the response to the pH1N1 vaccine in subjects with SMID. We evaluated immunogenicity by antibody response only. The vast majority of clinical studies on influenza vaccination focus on the antibody response, and cellular immunity is much less frequently investigated [18]. However, the cell-mediated immunity to pH1N1 vaccines also needs to be investigated to clarify the mechanism of diminished response to vaccination among subjects with SMID.

5. Conclusion

The results of our study indicate a diminished immune response to pH1N1 vaccine among subjects with SMID, though it was safe in this population. The first dose, and also the second dose of unadjuvanted vaccine, could not induce a large enough antibody response to meet the EMEA and FDA criteria. Consequently, subjects with SMID should be considered as immunosuppressed even after vaccination. More cautious observation and early treatment during the influenza season are needed for these subjects to avoid severe complications. In addition, further study is required to evaluate whether vaccination over consecutive influenza seasons can improve immunogenicity in SMID subjects.

Acknowledgments

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References

[1] World Health Organization. New influenza A/H1N1 virus: global epidemiological situation, June 2009. Wkly Epidemiol Rec 2009;84:249–57.
[2] Louie JK, Acosta M, Winter K, Jean C, Gavali S, Schechter R, et al. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. JAMA 2009;302:1896–902.

[3] 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.
[4] Helferty M, Vachon J, Tarasuk J, Rodin R, Spika J, Pelletier L. Incidence of hospital admissions and severe outcomes during the first and second waves of pandemic (H1N1) 2009. CMAJ 2009;182:1981–7.
[5] Harper SA, Bradley JS, Englund JA, File TM, Gravenstein S, Hayden FG, et al. Seasonal influenza in adults and children—diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 2009;48:1003–32.
[6] Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, et al. Influenza-associated hospitalizations in the United States. JAMA 2004;292:1333–40.
[7] Girard MP, Tam JS, Assossou OM, Kieny MP. The 2009 A (H1N1) influenza virus pandemic: a review. Vaccine 2010;28:4895–902.
[8] Mackay HJ, McGee J, Villa D, Gubbay JB, Tinker LM, Shi L, et al. 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.
[9] Lu CC, Wang YC, Lai JH, Lee TS, Lin HT, Chang DM. A/H1N1 influenza vaccination in patients with systemic lupus erythematosus: safety and immunity. Vaccine 2011;29:444–50.
[10] Labriola L, Hombrouck A, Marechal C, Van Gucht S, Brochier B, Thomas I, et al. Immunogenicity of an adjuvanted 2009 pandemic influenza A (H1N1) vaccine in haemodialysed patients. Nephrol Dial Transpl 2011;26:1424–8.
[11] 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.
[12] 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.
[13] 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.
[14] Hirst GK. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. J Exp Med 1942;75:49–64.
[15] World Health Organization. WHO manual on animal influenza diagnosis and surveillance; 2002.
[16] Committee for Proprietary Medical Products. Note for guidance on harmonisation of requirements for influenza vaccines. London: European Medicines Agency, Available from: <http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf>; 1996 [accessed 22.02.11].
[17] Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: clinical data needed to support the licensure of pandemic influenza vaccines, Available from: <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074786.htm>; May 2007 [accessed 22.02.11].
[18] Dormitzer PR, Galli G, Castellino F, Golding H, Khurana S, Del Giudice G, et al. Influenza vaccine immunology. Immunol Rev 2011;239:167–77.
[19] Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. N Engl J Med 2009;361:2405–13.
[20] Plennevaux E, Sheldon E, Blatter M, Reeves-Hoche 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.
[21] 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.
[22] Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. N Engl J Med 2009;361:2424–35.
[23] Kung HC, Huang KC, Kao TM, Lee YC, Chang FY, Wang NC, et al. A clinical study to assess the immunogenicity and safety of a monovalent 2009 influenza A (H1N1) vaccine in an area with low-level epidemics of pandemic influenza. Vaccine 2009;28:7337–43.
[24] Zhu FC, Wang H, Fang HH, Yang JG, Lin XJ, Liang XF, et al. A novel influenza A (H1N1) vaccine in various age groups. N Engl J Med 2009;361:2414–23.
[25] Frasca D, Diaz A, Romero M, Landin AM, Phillips M, Lechner SC, et al. Intrinsic defects in B cell response to seasonal influenza vaccination in elderly humans. Vaccine 2010;28:8077–84.
[26] McElhaney JE, Effros RB. Immunosenescence: what does it mean to health outcomes in older adults? Curr Opin Immunol 2009;21:418–24.
[27] 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.
[28] 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.
[29] 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.
[30] Perez-Padilla R, Fernandez R, Garcia-Sancho C, Franco-Marina F, Aburto O, Lopez-Gatell H, et al. Pandemic (H1N1) 2009 virus and Down syndrome patients. Emerg Infect Dis 2010;16:1312–4.
[31] Ram G, Chinen J. Infections and immunodeficiency in Down syndrome. Clin Exp Immunol 2011;164:9–16.

Cost-effectiveness of Hepatitis B Serology Testing Before Hepatitis B Vaccination in the Akha Tribal Children in Northeastern Thailand

To the Editors:

The inclusion of hepatitis B virus (HBV) vaccine in Thailand's national expanded program on immunization in 1992 has resulted in decreasing prevalence of HBV infection.[1] However, several tribal people who have migrated from China and Tibet, because they are not considered Thai nationals, are not eligible for healthcare services, including expanded program on immunization. Little is known about HBV prevalence and immunization rates in this population.

This report presents the seroprevalence of HBV infection and determines the cost-effectiveness of screening before vaccination in Akha tribal children. Preimmunization screening is not routinely recommended but maybe considered if it is cost effective.[2] For preimmunization screening to be considered cost effective, the cost of screening has to be less than or equal to the cost of vaccinating individuals already immune.[3]

In March 2010, the investigators were provided with de-identified data showing results of Hepatitis B surface antigen (HBsAg) and antibody (HBsAb) tests performed on 104 Akha children. Test for antibody to Hepatitis B core antigen (HBcAb) was not available. Children included in this report receive basic medical services at the Golden Triangle Rescue Mission in Chiang Rai province, Thailand. Lacking birth records, the investigators estimate these children to between the age of 5 and 17 years.

Assuming the 3-dose HBV vaccine recommendation would be completed, the investigators used the cost-effectiveness formula: $T \leq P \times V$, where T is the cost of serologic testing, P is the prevalence of children who were immune and/or infected, and V is the cost of vaccination.[3] The cost of screening (HBsAg and HBsAb) was $4.83 and the cost of 3-dose HBV vaccine series purchased in the open market was $23.48. The University of Florida Institutional Review Board approved this study.

Of the 104 children, 40 (38.5%) were HBsAg positive indicating either an acute or a chronic infection; and 7 (6.7%) were HBsAb positive, likely due to resolved infection and unlikely due to previous vaccination. The remaining 57 (54.8%) children were considered at risk for HBV infection.

With preimmunization screening, 47 children were determined not needing vaccination which resulted in a cost saving of $1103.56—a value much greater than the cost of $502.32 for screening the 104 children. With this high seroprevalence (~45%), it was cost effective to conduct preimmunization screening. The cost-effectiveness was lost at seroprevalence of <22%.

As this report suggests, in areas in which HBV infection is highly endemic, preimmunization screening may be cost effective until such time when successful routine hepatitis B immunization program for infants decrease HBV infections to break-even prevalence. The World Health Organization and the Centers for Disease Control and Prevention prefer HBcAb serology when single test is used as it identifies people with infection.[2,3] Alternatively, if HBsAb is used to detect immunity in people with possible previous infection, HBsAg testing must also be done to identify acute or chronic infection. Regardless of situation, endemicity, and test of choice, preimmunization serological testing provides an opportunity to identify patients needing further medical care and avoids unnecessary vaccination.

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REFERENCES

1. Chongsrisawat V, Yoocharoen P, Theamboonlers A, et al. Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization. *Trop Med Int Health*. 2006;11:1496–502.
2. World Health Organization. Hepatitis B Vaccine WHO position paper. Available at: http://www.who.int/wer/2009/wer8440.pdf. Accessed December 13, 2010.
3. Centers for Disease Control and Prevention. A comprehensive immunization strategy to eliminate transmission of Hepatitis B virus infection in the United States. *MMWR*. 2006;55(RR-16):1–40.

Alice in Wonderland Syndrome Caused by the 2009 Pandemic H1N1 Influenza A Virus

To the Editors:

We reported a case of Alice in Wonderland syndrome caused by the 2009 pandemic H1N1 influenza virus, confirmed by elevated antibody titers on a hemagglutinin inhibition assay. The case was a 5-year-old girl. She was previously healthy and had no history of convulsions or headache. She visited her family doctor complaining of a high fever on the second day of sickness in September 2009 and was diagnosed with influenza A infection proved by a rapid test for influenza. She was given oseltamivir for 3 days, and her clinical signs and symptoms disappeared after the initiation of therapy on the seventh day. She complained of micropsia and macropsia. The micropsia and macropsia were subjective complaints, and an examination by our ophthalmologist revealed no abnormality. Neurologic studies including EEG, brain magnetic resonance imaging (3 Tesla) followed by single-photon emission computed tomography showed normal results. There was no recent history of Epstein-Barr virus (EBV), chickenpox, or intestinal viral infection.

Laboratory tests on admission revealed a white blood cell count of 9100/μL (44.1% segmented, 6.7% eosinophils, 5.2% monocytes, 43.7% lymphocytes, 0.3% basophils, and no atypical lymphocytes), hemoglobin 14.0 g/dL, and platelet count 319,000/μL. The serum glucose level was 85 mg/dL, aspartate aminotransferase 36 U/L, alanine aminotransferase 17 U/L, sodium 138 mmol/L, potassium 4.6 mmol/L, and chlorine 106 mmol/L. Biochemical markers for a severity of inflammation revealed a normal C-reactive protein (<0.1 mg/dL) with normal levels of lactic dehydrogenase and creatinine kinase. A routine urinalysis showed normal results. Serologic studies revealed EBV-IgM 0.8 (normal: approximately 0.5) and EBV-IgG 0.5 (normal: approximately 0.5), cytomegalovirus-IgM 0.32 (normal: approximately 0.79), and cytomegalovirus-IgG 26.9 (normal: approximately 1.9). Laboratory blood investigations a month after the first visit to the

family doctor revealed a hemagglutinin inhibition titer to the 2009 pandemic H1N1 influenza A virus >1:1024. The visual abnormalities disappeared spontaneously within 2 months.

Alice in Wonderland syndrome is characterized by visual sensory abnormalities including micropsia and macropsia and they are regarded as a reflection of migraine or psychologic diseases. On the other hand, there have been several reports of this syndrome in cases with viral infections such as EBV,[1] varicella,[2] or coxsackie B1 virus.[3] A case of Alice in Wonderland syndrome associated with H1N1 influenza was reported,[4] while we were preparing this manuscript.

The neurologic prognosis of Alice in Wonderland syndrome caused by several viral infections is good.[2–4] Thus, Alice in Wonderland syndrome is completely different from encephalopathy caused by the 2009 pandemic H1N1 influenza.[5] Also, this syndrome seemed to have no relation to neuropsychiatric side effects of neuraminidase inhibitors such as osertamivir or zanamivir. No cases of Alice in Wonderland syndrome attributable to these drugs have been reported in Japan where neuraminidase inhibitors are common medications for seasonal influenza infection. The clear mechanism for a correlation of Alice in Wonderland syndrome to the 2009 pandemic H1N1 influenza remains to be elucidated.

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REFERENCES

1. Copperman SM. "Alice in Wonderland" syndrome as a presenting symptom of infectious mononucleosis in children: a description of three affected young people. *Clin Pediatr (Phila).* 1977;6:143–146.
2. Soriani S, Faggioli R, Scarpa P, et al. Alice in Wonderland syndrome and varicella. *Pediatr Infect Dis J.* 1998;17:935–936.
3. Wang SM, Liu CC, Chen YJ, et al. Alice in Wonderland syndrome caused by coxsackie virus B1. *Pediatr Infect Dis J.* 1996;15:470–471.
4. Augarten A, Aderka D. Alice in Wonderland syndrome in H1N1 influenza: case report. *Pediatr Emerg Care.* 2011;27:120.
5. Kedia S, Stroud B, Parsons J, et al. Pediatric neurological complications of 2009 pandemic influenza A (H1N1). *Arch Neurol.* 2011;68:455–462.

Moniliformis moniliformis Infection in Two Florida Toddlers

To the Editors:

Moniliformis moniliformis (thorny headed worm) is 1 of the 5 Acanthocephalan species widely distributed throughout the world. This helminth resides in the intestines of rodents, dogs, and cats, which serve as its definitive hosts. Infection with *M. moniliformis* is spread via intermediary hosts, primarily cockroaches, and beetles.

Human infection with this organism is rare. The last reported case of human infection with *M. moniliformis* in the United States was in a boy from Pensacola, FL in 1989.[1] We report 2 cases of *M. moniliformis* infection in central Florida.

The first patient was a 23-month-old otherwise healthy girl with worms in her stool for 2 months. She was otherwise asymptomatic. She was treated for a presumed tapeworm infection with mebendazole, however, the worms persisted. The patient's stool sample contained a visible cream-colored worm which was approximately 20-cm long. The worm was segmented, with a beaded appearance, and did not have the typical flattened appearance of a tapeworm. Oval-shaped eggs measuring 60 × 83 μm were seen on microscopic examination. Based upon the morphology and measurement of the parasite and the ova, the worm was identified as *M. moniliformis*. Animal exposures included a dog and cat. She frequently put items, including insects, found on floor into her mouth.

She was treated with three 11-mg/kg doses of pyrantel pamoate separated by 2-week intervals. No worms were seen after the third dose. She remains asymptomatic.

The second patient was a 15-month-old girl from central Florida who also experienced asymptomatic passage of worms in the stool, identified as *M. moniliformis* (Figure). Animal exposures included a pet chinchilla as well as chickens, cows, dogs, cats, and young squirrels at her grandparent's farm. Her parents and grandparents had witnessed her putting cockroaches in her mouth on several occasions. The child was also was treated with mebendazole, followed by pyrantel pamoate after identification of the organism was made. No more worms were seen in the stool after treatment. The patient has continued to do well with no symptoms.

Human infection with any of the Acanthocephalan worms is rare, but is most commonly caused by infection with *M. moniliformis* and *Macracanthorhyncus hirudinaeceus*.[2] Acanthocephalan worms possess unique physical characteristics helpful in identification. They lack a digestive tract, a coelom, and a circulatory system. They are also distinguished by their spinous retractile proboscis ("thorny head"), which enables attachment to the intestinal wall, and by their spindle shaped, pseudo-segmented body. Adult female worms measure 100 to 270 mm and males measure 40 to 130 mm. Their thick-walled, oval shaped eggs measure 85 to 118 × 40 to 52 μm.[3]

A wide range symptoms have been described, including diarrhea, weight loss, irritability, edema, weakness,[4,5,6] and asymptomatic passage of worms.[1,3,7] In many cases, there is a history of ingestion of a beetle, or a cockroach. Many cases are described in toddlers, who may be at particular risk because of their propensity to put nonfood objects into their mouths.

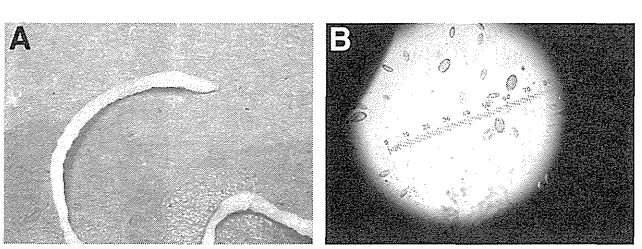

FIGURE 1. A, Macroscopic view of *M. moniliformis* from stool sample of patient 2. B, Microscopic view of *M. moniliformis* eggs from stool sample of patient 2.

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REFERENCES

1. Counselman K, Field C, Lea G, et al. *Moniliformis moniliformis* from a child in Florida. *Am J Trop Med Hyg.* 1989;41:88–90.
2. Neafie RC, Marty AM. Unusual infections in humans. *Clin Microbiol Rev.* 1993;6:34–56.
3. Sahar MM, Madani TA, Al Moshen IZ, et al. A child with an acanthocephalan infection. *Ann Saudi Med.* 2006;26:321–324.
4. Salehabadi A, Mowlavi G, Sadjjadi SM. Human infection with *Moniliformis moniliformis* (Bremser 1811) (Travassos 1915) in Iran: another case report after three decades. *Vector Borne Zoonotic Dis.* 2008;8:101–103.
5. Berenji F, Abdolmajid F, Hosseininejad Z. A case of *Moniliformis moniliformis* (Acanthocephala) infection in Iran. *Korean J Parasitol.* 2007; 45:145–148.
6. Moayedi B, Maleki IM, Ghadirian E. Human infections with *Moniliformis moniliformis* (Bremser 1811) Travassos 1915 (Syn. Moniliformis dubius) report of a case in Isfahan, Iran. *Am J Trop Med Hyg.* 1971;20:445–448.
7. Prociv P, Walker J, Crompton LJ, et al. First record of human acanthocephalan infection in Australia. *Med J Aust.* 1990;152:215–216.

Severe Myelosuppression and MRSA Infection as the Presentation of Perinatal HIV Infection

To the Editors:

The association between methicillin-resistant *Staphylococcus aureus* (MRSA) and human immunodeficiency virus (HIV) has been well described in adults. In recent years, there has been a significant increase in the incidence in MRSA infections, including skin and soft-tissue infections, in children and adolescents.[1] Two cases of adults with severe MRSA infection as the initial presentation of acquired immunodeficiency syndrome (AIDS) have been reported,[2,3] whereas no cases have yet been described in the pediatric age group. We describe an infant diagnosed with perinatal HIV infection after presenting with multiple community-associated MRSA (CA-MRSA) abscesses and concomitant severe neutropenia and anemia.

A 9 month-old African American female child was hospitalized with buttock abscesses that had progressively worsened during a 4-week period, even with amoxicillin-clavulanate therapy. She was born by uncomplicated spontaneous vaginal delivery at term, and the mother's first trimester HIV enzyme immunoassay was negative. The infant was formula-fed and had reached all developmental milestones. Her medical history was significant for hospitalization at the age of 2 weeks for fever and sepsis evaluation, hospitalization at the age of 2 months for pneumonia treated with a beta-lactam antibiotic, and iron-deficiency anemia. During the hospitalization at the age of 2 weeks, she was treated with aminoglycoside and beta-lactam antibiotics; the hemoglobin, white blood cell (WBC) count, and absolute neutrophil count (ANC) were within normal limits (15.1 g/dL, 7500 cells/μL, and 3000 cells/μL, respectively).

At the current presentation, the infant had multiple deep wounds over the buttocks with healthy-appearing granulation tissue present after undergoing incision and drainage. She then developed fever to 40.4°C that prompted further evaluation. Laboratory results were significant for hemoglobin 5.4 g/dL and WBC count of 5400 cells/μL with ANC 100 cells/μL. Platelet count was 259,000 cells/μL. Wound culture grew MRSA, susceptible to both clindamycin and erythromycin. Treatment with clindamycin was continued along with local wound care. No other signs of systemic MRSA infection were identified, and several blood cultures obtained during hospitalization were negative. Workup of the anemia was consistent with anemia of chronic disease. One blood transfusion of 10 mL/kg packed red blood cells was administered on admission with subsequent rise in hemoglobin to 10.6 g/dL.

The infant was further evaluated for immunodeficiency due to persistent neutropenia and fever despite appropriate antibiotic therapy. Quantitative immunoglobulins revealed an elevated IgG level (2334 mg/dL). The CD4 cell count (1186 cells/μL, 22%) and CD4/CD8 ratio (0.5) were both low for age. Bone marrow aspirate revealed few erythroid precursors and rare immature neutrophils. Granulocyte-macrophage colony stimulating factor (GM-CSF) was started on hospital day 5. HIV-1 DNA PCR was positive on 2 separate measurements, and viral load measured by HIV-1 RNA PCR was 181,000 copies/mL. HIV genotype revealed no resistance mutations. *Pneumocystis jiroveci* prophylaxis with trimethoprim-sulfamethoxazole was started, and a highly active antiretroviral therapy (HAART) regimen including lopinavir/ritonavir, zidovudine, and lamivudine was initiated on hospital day 22 because of persistent neutropenia. The ANC remained below 1000 cells/μL after receiving 17 days of GM-CSF but resolved 1 day after HAART was initiated, with WBC count 7300 cells/μL and ANC 3070 cells/μL. A 4-week course of clindamycin was completed. After 14 months of HAART, the viral load remained undetectable (<50 copies/mL) with an increase in absolute CD4 cell count (1513 cells/μL, 42%). ANC remained stable above 1000 cells/μL. Both parents were subsequently diagnosed with HIV infection.

Based on the clinical presentation and antibiotic susceptibilities of the MRSA isolate, the infection would be epidemiologically defined as CA-MRSA, even with a history of prior hospitalization.[4] The severity of clinical presentation and the length of recovery from infection were unusual, and it is likely that the neutropenia played an important role in the immunosuppressed state. Neutropenia is correlated with increased risk for *Staphylococcus aureus* colonization and infection in HIV-infected individuals.[5,6] In the previously mentioned adult case studies, neutropenia was not specifically identified as a risk factor; however, both patients were described as severely immunocompromised. In the first case, a 35-year-old Hispanic man with previously undiagnosed AIDS presented with MRSA necrotizing fasciitis. The work-up was significant for WBC count 1600 cells/μL, CD4 cell count 20 cells/μL, viral load 358,000 copies/mL, but ANC was not reported.[2] In the second case, a 29-year-old Hispanic man presented with septic pulmonary emboli associated to a recent CA-MRSA skin and soft-tissue infection. The immunodeficiency work-up revealed WBC count 3500 cells/μL, CD4 cell count 2 cells/μL and HIV seropositivity; ANC and viral load were not reported.[3]

Several case reports document myelosuppression as the presentation of HIV infection in children, but none were associated with MRSA infection. In cases of isolated anemia or thrombocytopenia, either HIV infection or an autoimmune process was the presumed etiology for the hematologic aberration.[7–10] With HAART, improvement in the hematologic abnormality occurred.[9] In another case, a 7-year-old boy was diagnosed with acute myelogenous leukemia (AML) and concurrent HIV infection.[11] A mild anemia with hemoglobin 8.9 g/dL, WBC count 5200 cells/μL, and plate-

let count 21,000 cells/μL were reported. The authors indicate the occurrence of AML and HIV may have been coincidental but speculated that HIV infection may also have played an etiologic role in AML by defective T cell control of hematopoiesis or failure of immunosurveillance. An additional case was reported in a 17-year-old adolescent with a 4-year history of neutropenia and repeated infections, who presented with severe dysphagia and progressive peroneal nerve palsy.[12] The WBC count was 1530 cells/μL, ANC 382 cells/μL, absolute CD4 cell count 2 cells/μL, and viral load 559,000 copies/mL. HIV-related cytomegalovirus esophagitis and polyneuropathy were diagnosed.

Suppression of bone marrow as a direct consequence of HIV infection may occur as a result of abnormal cytokine expression and alteration of bone marrow microenvironment.[13] Worsening HIV disease indices, such as CD4 cell count less than 200 cells/μL and HIV viral load greater than 100,000 copies/mL have been associated with the development of neutropenia in adults while resolution of neutropenia was associated with the use of HAART and CD4 cell count greater than 500 cells/μL.[14] Therapeutic options for neutropenia have additionally included the use of granulocyte colony stimulating factor (G-CSF) or GM-CSF.[15] However, in our case, the infant's neutropenia was refractory to GM-CSF alone. This observation suggests that the neutropenia was most likely due to the intrinsic myelosuppressive effects of HIV infection.

The myelosuppressive effects of HIV are striking as part of the initial presentation of perinatal HIV infection with severe HIV-associated neutropenia likely increasing susceptibility to serious CA-MRSA infection. Clinical resolution of the CA-MRSA infection resulted from the combined approach of appropriate antibiotic therapy, surgical drainage, and initiation of HAART.

Finally, our case illustrates a missed opportunity for prevention of perinatal HIV infection. The Centers for Disease Control and Prevention issued updated guidelines in 2006 for HIV screening in the first and third trimesters of pregnant women with high-risk behavior and those living in HIV high-incidence states.[16] Lack of prenatal care and failure to repeat HIV testing in late pregnancy contribute to more than half the cases of HIV-infected infants.[17] A negative HIV antibody test in a pregnant woman in the first trimester does not exclude the possibility of perinatal HIV transmission.

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REFERENCES

1. Srinivasan A, Seifried S, Zhu L, et al. Methicillin-resistant *Staphylococcus aureus* infections in children and young adults infected with HIV. *AIDS Res Hum Retrovir*. 2009;25:1219–1224.
2. Olsen RJ, Burns KM, Chen L, et al. Severe necrotizing fasciitis in an HIV-positive patient caused by methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol*. 2008;46:1144–1147.
3. Kruse BT, Vadeboncoeur TF. Methicillin-resistant *Staphylococcus aureus* sepsis presenting with septic pulmonary emboli. *J Emerg Med*. 2009;37:383–385.
4. David MZ, Glikman D, Crawford SE, et al. What is community-associated methicillin-resistant *Staphylococcus aureus*. *J Infect Dis*. 2008;197:235–243.
5. Weinke T, Schiller R, Fehrenbach FJ, et al. Association between *Staphylococcus aureus* nasopharyngeal colonization and septicemia in patients infected with the human immunodeficiency virus. *Eur J Clin Microbiol Infect Dis*. 1992;11:985–989.
6. Nguyen MH, Kauffman CA, Goodman RP, et al. Nasal carriage of and infection with *Staphylococcus aureus* in HIV-infected patients. *Ann Intern Med*. 1990;130:221–225.
7. Labrune P, Blanche S, Catherine N, et al. Human immunodeficiency virus-associated thrombocytopenia in infants. *Acta Paediatr Scand*. 1989;78:811–814.
8. Morad AB, Steuber CP, Mahoney DH, et al. Hypoplastic anemia in an infant with human immunodeficiency virus (HIV) infection. *Am J Hematol*. 1993;42:236.
9. Rheingold SR, Burnham JM, Rutstein R, et al. HIV infection presenting as severe autoimmune hemolytic anemia with disseminated intravascular coagulation in an infant. *J Pediatr Hematol Oncol*. 2004;26:9–12.
10. Verma S, Gupta P. HIV infection masquerading as idiopathic thrombocytopenic purpura. *Indian J Pediatr*. 2008;74:85.
11. Tullu MS, Date NB, Ghildiyal RG, et al. Acute myelogenous leukemia in a child with HIV infection. *Eur J Pediatr*. 2010;169:629–631.
12. Sternfeld T, Weidenbach H, Lorenzen S, et al. A 17-year-old adolescent with chronic neutropenia, recurrent infections, severe dysphagia and peroneus palsy. *Internist (Berl)*. 2009;50:1018–1021.
13. Coyle TE. Hematologic complications of human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *Med Clin North Am*. 1997;81:449–470.
14. Levine AM, Karim R, Mack W, et al. Neutropenia in human immunodeficiency virus infection. *Arch Intern Med*. 2006;166:405–410.
15. Evans RH, Scadden DT. Haematological aspects of HIV infection. *Baillieres Best Pract Res Clin Haematol*. 2000;13:215–230.
16. Centers for Disease Control and Prevention. Revised recommendations for hiv testing of adults, adolescents, and pregnant women in health-care settings. *MMWR Recomm Rep*. 2006;55(RR-14).
17. Steele RW. Late pregnancy screening for human immunodeficiency virus. *Pediatr Infect Dis J*. 2011;30:72–74.

Immunogenicity of a Monovalent 2009 Influenza A (H1N1) Vaccine Among Pregnant Women: Lowered Antibody Response by Prior Seasonal Vaccination

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Background. Pregnant women are a high-risk group for influenza-associated complications and hospitalizations.

Methods. To examine the immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine among pregnant women, a prospective cohort study was performed at 2 medical institutes of obstetrics in Japan. One hundred fifty subjects received 2 subcutaneous doses of vaccine 3 weeks apart. The hemagglutination inhibition antibody titer was measured in serum samples collected at 3 time points: before vaccination, 3 weeks after the first dose, and 4 weeks after the second dose.

Results. The first dose of vaccine induced a ⩾10-fold rise in the average level of antibody. The seroresponse rate (⩾4-fold rise) was 91%, and the seroprotection rate (postvaccination titer ⩾1:40) was 89%. The second dose of vaccine conferred little additional induction of antibodies. Similar immune responses were observed irrespective of body mass index before pregnancy, trimester, or age at vaccination. However, lesser immune response was shown in subjects who had received the 2009–2010 seasonal influenza vaccine before the H1N1 vaccination.

Conclusions. A single dose of vaccine induced an adequately protective level of immunity in pregnant women. The potential interference with seasonal vaccination requires a more thorough investigation to prepare for future influenza pandemics.

Pregnant women are a high-risk group for influenza-associated complications and hospitalizations. Among healthy pregnant women, excess deaths were documented during the influenza pandemics of 1918–1919 and 1957–1958 [1–3]. Higher hospitalization rates among pregnant women were also reported in the 2009 influenza A (H1N1) pandemic [4, 5]. Even in nonpandemic influenza seasons, hospitalization rates were increased in all trimesters of pregnancy [6, 7] and were particularly higher in the third trimester or among women with underlying illnesses [7–10]. Therefore, the control of influenza among pregnant women is one of the most important challenges in public health.

Influenza vaccination is the most effective method for preventing influenza illness and its complications. The World Health Organization guidelines that were prepared for the 2009 influenza A (H1N1) pandemic placed pregnant women in the highest priority group to receive vaccination. Therefore, the Japanese government revised the package insert for influenza vaccine, which had originally indicated that pregnancy was a contraindication for vaccination, and advised pregnant women to receive the vaccination.

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