厚生労働行政推進調査事業費
（肝炎等克服緊急対策研究事業）

肝炎ウイルスの新たな感染防止
－残された課題・今後の対策－

平成 30 年度　総括・分担報告書

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研究報告

肝炎ウイルスの新たな感染防止 －残された課題・今後の対策－

研究代表者 **四柳 宏** 東京大学医科学研究所先端医療研究センター感染症分野 教授

研究要旨

肝炎ウイルスの感染を集団レベルでコントロールするためには多面的なアプローチが必要である。本研究班の目標として（1）一般生活者・保育施設勤務者・医療従事者を対象とした e-learning system の構築、（2）HB ワクチンの接種状況・感染状況に関する調査、（3）急性肝炎の発生状況に関する正確な状況把握の検討、を掲げた。

本年度は、（1）e-learning system を構築し、次年度に保育関係者、医療従事者を対象に試行する予定となっている （2）①全国の医療施設における実態把握のためアンケート調査を開始した ②医療従事者に対する HB ワクチン接種後の HBV への感染状況、ワクチンの追加接種の効果を検証するシステムを構築した ③ HB ワクチン定期接種後の効果と導入後の新規感染を把握するための準備を行った （3）① 2018 年度にアウトブレイクを起こした A 型肝炎の実態把握を行った ②ビッグデータを用いた C 型肝炎の家族内伝播の予備調査を行った など計画に従って研究を推進している。

A. 研究目的

肝炎対策基本法には“肝炎対策基本指針”が定められており、この中の一つに“肝炎に関する啓発及び知識の普及並びに肝炎患者等の人権の尊重に関する事項”が挙げられている。

本研究班は“肝炎に関する啓発及び知識の普及”を目標にしている。同時に肝炎対策基本指針の中に定められている“肝炎の予防のための施策に関する事項”に関する研究を行うことも目的にしている。

B. 研究方法

本研究班の目標として（1）一般生活者・保育施設勤務者・医療従事者を対象とした e-learning system の構築、（2）HB ワクチンの接種状況・感染状況に関する調査、（3）急性肝炎の発生状況に関する正確な状況把握の検討、を掲げた。

C. 研究結果

（1）一般生活者・保育施設勤務者・医療従事者を対象とした e-learning system の構築

・四柳宏研究代表者

e-learning に加えウイルス肝炎の感染経路に関する Q and A を他の研究班と共同で作成した。

・江口有一郎研究分担者

班員の協力のもと、「一般生活者」「老人施設関係者」に対するガイドラインについて、パワーポイントスライドおよび音声ガイドからなる動画コンテンツを作成し、次年度に調査を行う体制を整えた。

・八橋弘研究分担者

看護学生 670 名を含む病院職員 5330 名を対象としてウイルス肝炎の感染経路及び感染確率に関する理解度を明らかにする目的で実施した無記名アンケート調査の結果を解析し、感染経路の理解に関する問題点を明らかにした。

・森屋恭爾研究分担者

医療の場における肝炎ウイルス感染予防の事態を知るため、日本病院会に加盟している組織に対するアンケート調査を計画した。倫理審査を通過した後アンケートを実施した。

(2) HB ワクチンの接種状況・感染状況に関する調査

・細野覚代研究分担者

全国の病院において医療関係者を対象とした肝炎ウイルス検査データおよび HBV 感染予防状況のデータベース構築、サーバーへの登録の準備を進めた。

・田中靖人研究分担者

細野研究分担者と協力して医療関係者を対象とした肝炎ウイルス検査データおよび HBV 感染予防状況の実態調査を行い、データベースを構築する作業を行った。

B 型肝炎ワクチン（HB ワクチン）定期接種化以前に出生した小児の B 型肝炎感染疫学の調査として、エコチル調査・愛知ユニットセンターに登録された 8 歳学童期調査および 8 歳詳細調査の参加者を対象に HBV 感染の実態調査を行う準備を行った。

・高野智子研究分担者

保育現場におけるガイドライン（『保育の場において血液を介して感染する病気を防止するためのガイドライン－ウイルス性肝炎の感染予防を中心に－』）の理解度及び感染対策の実際を検証するために、大阪市内の保育施設勤務者にアンケート調査を行った。

・酒井愛子研究分担者

小児における B 型肝炎ウイルスの感染実態および B 型肝炎ワクチン定期接種開始後のワクチン接種率・HBs 抗体獲得率・HBs 抗体持続期間を明らかにするため、病院受診者の残余検体を用いた多施設共同疫学調査の倫理申請を行った。

・森岡一朗研究分担者

酒井研究分担者と協力して筑波大学を主研究機関としたグループを結成し、2019 年度からの本研究の遂行に向けて、日本大学医学部附属板橋病院および神戸こども初期急病センターの倫理委員会の承認を得て、研究体制を整えた。

・田中敏博研究協力者

静岡県における HB ワクチン接種後の HBs 抗体追跡調査（多施設共同研究）に必要な準備作業を行った。

(3) 急性肝炎の発生状況に関する正確な状況把握の検討

・相崎英樹研究分担者

本年流行した A 型急性肝炎に関して感染症サーベイランス事業の結果と定点医療施設の観察結果と比較した。さらに、A 型急性肝炎の米国における状況と対策を解析した。

・田倉智之研究分担者

医療ビッグデータを応用し、C 型肝炎を対象に抽出・連結を行い、予備調査を実施した。

D. 考察

本年度は初年度であり、(1) ～ (3) の研究グループにおいて研究を円滑に行うための準備作業を行った。以下に今後の課題を挙げる。

(1) e-learning に関しては参加者が e-learning を行うことでどのようなことを学んだかの評価が必要である。これに関しては八橋研究分担者・江口研究分担者にも協力して頂き、問題やアンケートによる評価を考えている。

(2) 成人の HB ワクチンに関してはワクチン無効例への対策、ブースター接種の必要性の有無が大きな問題である。研究期間の間にできるだけ多くのことを明らかにする必要がある。小児に関して定期接種の効果を明らかにするにはかなりのサンプル数が必要でその確保が課題である。

(3) B 型肝炎・C 型肝炎はともに 5 類の全数届出感染症であるが、届出率は低い。この検討により今後どの程度が報告されているか、地域差はどうであるかなどが明らかにされることが期待される。根本的な対策の立案は容易でないが、届出がきちんと行われるための提言のようなものを考えていくべきである。

E. 結論

ウイルス肝炎のコントロールのための研究を3つのプロジェクトを中心に展開する準備を行なった。来年度以降実際の調査を行う予定である。

F. 健康危険情報

なし

G. 研究発表

各研究者の稿参照のこと

H. 知的所有権の取得状況（予定を含む）

1．特許取得
該当なし
2．実用新案登録
該当なし
3．その他
該当なし

感染防止のための正しい知識の取得の向上を目指した e-learning システムの構築に関する研究

研究分担者　江口有一郎　佐賀大学医学部附属病院　肝疾患センター　特任教授

研究要旨

【背景】厚生労働省研究班で作成した感染対策ガイドライン（一般生活者向け・保育施設勤務者向け・老人保健施設勤務者向け）について、利用者が学びやすい環境を構築し、肝炎ウイルスの感染防止に関する正しい知識を普及することが大切である。【方法】班員の協力のもと、「一般生活者」「老人施設関係者」に対するガイドラインについて、パワーポイントスライドおよび音声ガイドからなる動画コンテンツを作成した。構成は、基礎知識の解説および巻末に知識の取得状況を把握するための確認テストを盛り込んだ。【結果】約 5 分で感染防止に関する基礎知識を学習できる動画コンテンツを作成し、web に掲載した。【結語】。今後は作成した e-learning システムの利用促進を図り、システムの利用状況や利用者の知識習得状況等を解析し、正しい知識の普及を促進していく予定である。

A. 研究目的

本研究班の代表者が 2012 年度から 2014 年度まで主任研究者を務めた“集団生活の場における肝炎ウイルス感染予防ガイドラインの作成のための研究班”では一般生活者・保育関係者・老人施設関係者に対するガイドラインが作成されている。このガイドラインは厚生労働省・肝炎情報センターのウエブサイトに掲載され、活用されていることが期待されるが、その利用状況や知識の取得状況などは明らかになっていない。本研究では、利用者が学びやすい環境を構築し、肝炎ウイルスの感染防止に関する正しい知識を普及することを目的とし、ガイドライン毎の対象者にとって、ガイドラインの内容を学びやすい e-learning システムを構築し対象者への普及を図る。その上で、システムの利用状況や利用者の知識習得度に関する情報を収集・解析し、必要な課題の解決を図るなど、対象者の知識取得率向上のための取り組みを行うことを目的としている。

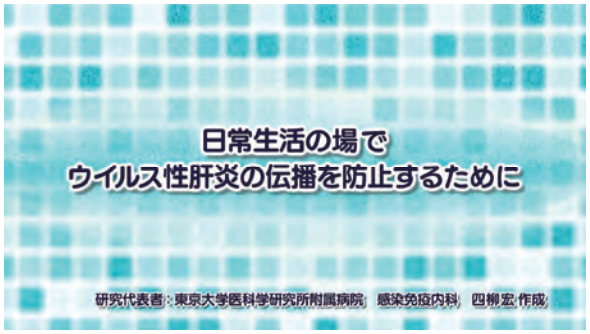
そのため今年度はまず e-learning システムを構築する。

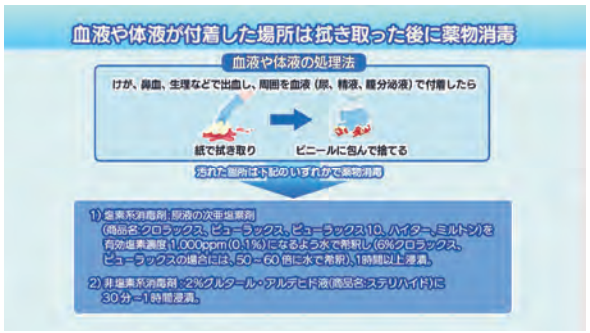
B. 研究方法

班員（四柳宏研究代表者･磯田広史研究協力者）の協力のもと、ガイドラインに書かれた内容をもとに「一般生活者」「老人施設関係者」に対するガイドラインについて、e-learning コンテンツ案を作成した。研究班の班会議で開示し、班員からの意見を踏まえコンテンツを完成させた。

C. 研究結果

作成した e-learning コンテンツを次に示す（図 1：一般生活者、図 2：老人施設関係者、図 3：共通の項目、図 4：確認テスト）。それぞれの e-learning コンテンツは、情報を教示する内容とナレーションで構成され、それぞれの内容に関する確認テストが最後に行われる。また、e-learning の内容は更新可能なシステムで運用されており、確認テストも内容の更新が可能である。また、受講者の属性や正答率をモニタリングすることにより、e-learning のコンテンツの更新へ反映させることができる様にシステムを構築した。





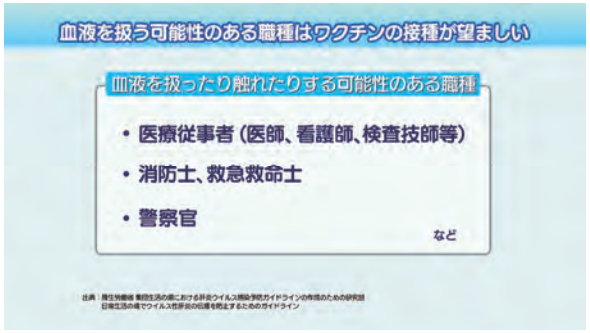


図１　「一般生活者」向けコンテンツ

図２　「老人施設関係者」向けコンテンツ

図３　両方のコンテンツに共通の項目

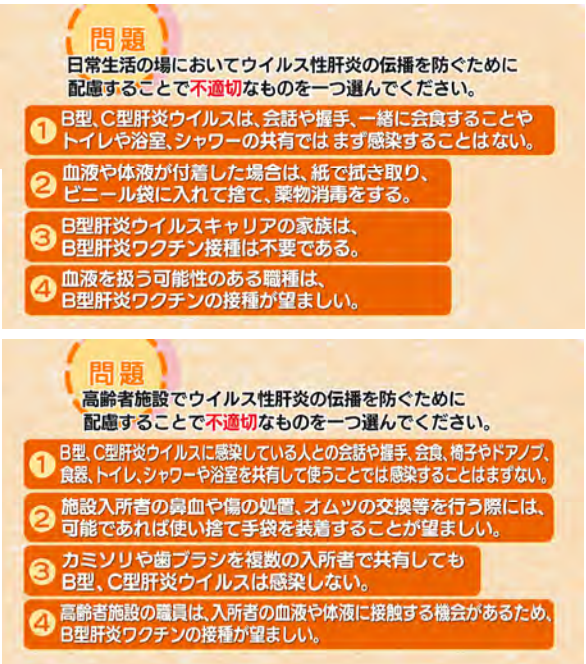


図４　確認テスト

D. 考察

「一般生活者」「老人施設関係者」に対するガイドラインについて、e-learning コンテンツを作成した。現在はテストサイトで運用しており、順次対象施設においてシステムの利便性の評価、利用率等の利用状況、利用者の知識習得度（合格率）といった点についてパイロットスタディを実施していく予定である。また、要望が多い情報についてはコンテンツの拡充についても検討を進める。

E. 結論

今年度の目標である e-learning システムのプロトタイプを作成した。引き続き現場での応用および全国展開を目指して研究を進めていく予定である。

F. 健康危険情報

なし

G. 研究発表

1．論文発表

なし

2．学会発表

なし

H. 知的所有権の取得状況（予定を含む）

1．特許取得

該当なし

2．実用新案登録

該当なし

3．その他

該当なし

看護学生と病院職員に対するウイルス肝炎の感染経路及び感染確率に関する理解度に関する調査報告

研究分担者 八橋 弘 独立行政法人国立病院機構長崎医療センター
臨床研究センター長
共同研究者 山﨑 一美 独立行政法人国立病院機構長崎医療センター
肝臓内科、臨床研究センター 室長

研究要旨

看護学生 670 名を含む病院職員 5330 名を対象としてウイルス肝炎の感染経路及び感染確率に関する理解度を明らかにする目的で実施した無記名アンケート調査の結果、以下の 3 点を明らかにした。

1. B 型肝炎は、血液を介して感染し空気感染しないということに対する理解度については、国家資格を有する者、医療従事者として患者に直接かかわる職種では、概ね正しく理解されていると考えられた。

2. E 型肝炎という疾患そのものが一般的には知られていない、正しく理解されていないと考えられた。

3. C 型肝炎が食事を介して感染するか否か、針刺し事故での感染確率、蚊を介して感染が成立するかに関する理解は、医師以外の職種では、概ね C 型肝炎の感染確率を過大評価していると考えられた。

A. 研究目的

厚生労働行政推進調査事業費補助金（肝炎等克服政策研究事業）『肝炎ウイルス感染者の偏見や差別による被害防止への効果的な手法の確立に関する研究』班（研究代表者：八橋弘）と厚生労働行政推進調査事業費補助金（肝炎等克服政策研究事業）『肝炎ウイルスの新たな感染防止・残された課題・今後の対策』研究班（研究代表者：四柳宏）とは相互に密に連絡し合い、連携して研究事業を推進している。

『肝炎ウイルス感染者の偏見や差別による被害防止への効果的な手法の確立に関する研究』班で実施した調査内容の中から、看護学生及び病院職員を対象としたウイルス肝炎の感染経路及びウイルス肝炎の感染性についての理解度に関して明らかにする目的で別途解析をおこなったので、その結果を報告する。

B. 研究方法

ウイルス肝炎の感染経路及びウイルス肝炎の感染性についての理解度に関するアンケート調査を実施した。11 問題、22 項目について問題集を作成し、解答後は直ちに正しい答えを理解できるように封印した解答集を問題集と合わせて配布することで、正しい知識、適切な対応を自己学習できるようにした。2018 年 8 月 2 日の倫理審査委員会の承認後に下記の研究協力施設に問題集と解説書を送付した。

29 の国立病院機構病院と国立国際医療センター病院に所属する 15772 名の病院職員と 16 の国立病院機構付属看護学校と看護大学校、看護大学に所属する 3962 名の看護学生、合わせて 19734 名を対象にアンケート用紙を配布した。2018 年 12 月 3 日の時点で 8242 名（41.8％）から回収でき、5330 名分のアンケート調査の中間解析をおこなった。

C. 研究結果

5330 名分のアンケート調査の中で年齢層が明記されていたのは 5149 名で、うち 18 歳から 22 歳は 902 名、23 歳から 30 歳は 1503 名、31 歳から 40 歳は 1245 名、41 歳以上は 1499 名であった（図 1）。

職種が明記されていたのは、看護学生 670 名、看護師 2694 名、医師 252 名、薬剤師 140 名、検査技師 183 名、放射線技師 135 名、事務職員 560 名、その他 506 名であった（図 1）。

B 型肝炎が咳をすることで感染するか否かの設問に対する正解率を算出すると、看護学生 71.7％、看護師 94.3％、医師 93.7％、薬剤師 98.6％、検査技師 96.7％、放射線技師 96.3％、事務職員 75.7％、その他 80.0％であった（図 2）。

E 型肝炎が食事を通じて感染する疾患であるかに関する設問に対する正解率を算出すると、看護学生 26.3％、看護師 29.7％、医師 87.3％、薬剤師 62.9％、検査技師 71.6％、放射線技師 18.5％、事務職員 15.0％、その他 19.0％であった（図 3）。

C 型肝炎患者と鍋料理を共にすることで感染する確率に関する設問に対する正解率を算出すると、看護学生 38.3％、看護師 65.2％、医師 92.5％、薬剤師 83.6％、検査技師 74.9％、放射線技師 69.6％、事務職員 41.6％、その他 48.2％であった（図 4）。

C 型肝炎の針刺し事故による感染確率に関する設問に対する正解率を算出すると、看護学生 7.6％、看護師 23.8％、医師 73.0％、薬剤師 37.9％、検査技師 48.9％、放射線技師 29.6％、事務職員 10.5％、その他 14.1％であった（図 5）。

C 型肝炎が蚊を媒体として感染する感染確率に関する設問に対する正解率を算出すると、看護学生 10.5％、看護師 30.1％、医師 61.9％、薬剤師 42.1％、検査技師 52.2％、放射線技師 34.8％、事務職員 19.6％、その他 26.6％であった（図 6）。

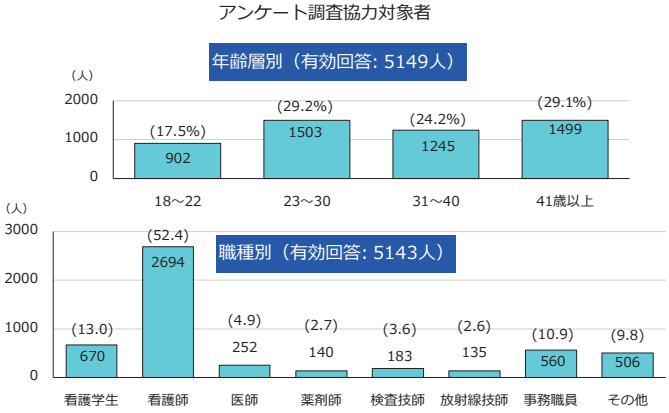


図 1　看護学生及び病院職員を対象としたウイルス肝炎全般、特にウイルス肝炎の感染性についての理解度に関するアンケート調査

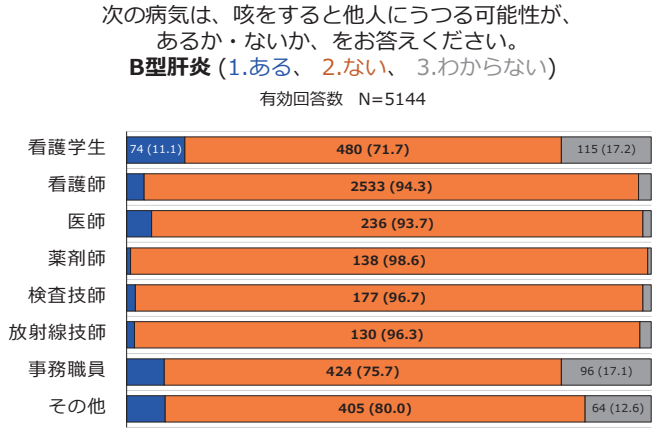


図 2　看護学生及び病院職員を対象としたウイルス肝炎全般、特にウイルス肝炎の感染性についての理解度に関するアンケート調査

次の病気は、食事を通じて感染する可能性が、
あるか・ないか、をお答えください。
E型肝炎 (1.ある、 2.ない、 3.わからない)

有効回答数 N=5142

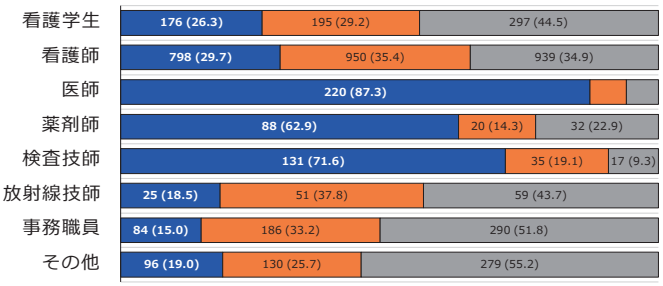


図３ 看護学生及び病院職員を対象としたウイルス肝炎全般、
特にウイルス肝炎の感染性についての理解度に関するアンケート調査

C 型肝炎の患者さんと一緒に鍋料理を食べることになりました。
食事をすることで、あなたが感染する確率はどれくらいであるか、
1 つ選んでください。

1. 0% / 2. 2%前後 / 3. 20%前後 / 4. 80%以上 / 5. わからない

有効回答数 N=5146

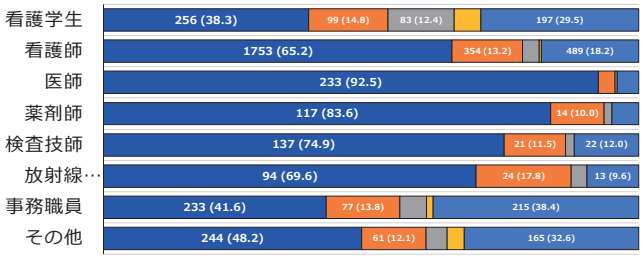


図４ 看護学生及び病院職員を対象としたウイルス肝炎全般、
特にウイルス肝炎の感染性についての理解度に関するアンケート調査

C 型肝炎の患者さんの採血をした針を誤って自分に刺してしまいました。
針刺しであなたが感染する確率はどれくらいであるか、
1 つ選んでください。

1. 0% / 2. 2%前後 / 3. 20%前後 / 4. 80%以上 / 5. わからない

有効回答数 N=5142

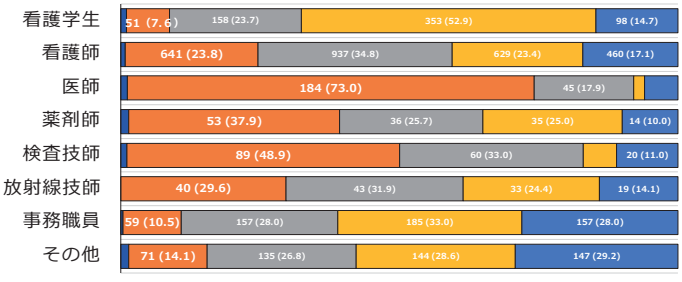


図５ 看護学生及び病院職員を対象としたウイルス肝炎全般、
特にウイルス肝炎の感染性についての理解度に関するアンケート調査

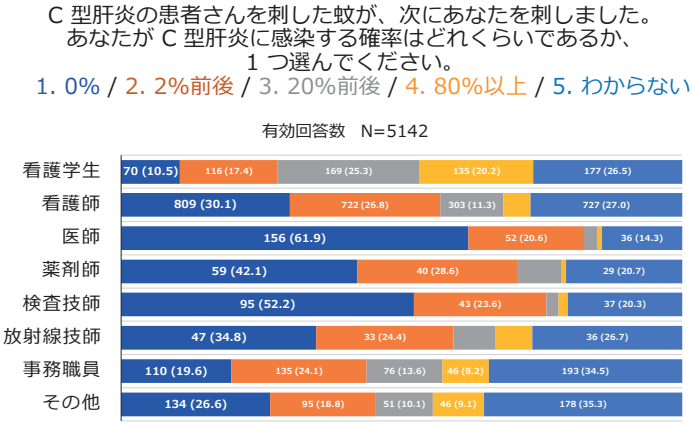


図 6　看護学生及び病院職員を対象としたウイルス肝炎全般、
特にウイルス肝炎の感染性についての理解度に関するアンケート調査

D. 考察

看護学生 670 名を含む病院職員 5330 名を対象としてウイルス肝炎の感染経路及び感染確率に関する理解度を明らかにする目的で、無記名アンケート調査の結果を実施した結果、以下の 3 点のことが明らかになった。

1. B 型肝炎は、血液を介して感染し、咳をすることなどでは感染しない、空気感染しないということに対する理解度は、看護学生や事務職員では 70%台の正解率であった。一方、看護師、医師、薬剤師、検査技師など病院職員の中でも国家資格を有する者の正解率は 93%以上であり、医療従事者として患者に直接かかわる職種では、B 型肝炎の感染経路について概ね正しく理解されていると考えられた。
2. E 型肝炎は、E 型肝炎ウイルスに汚染された水や食品を介して経口感染する感染症である。医師で 87.3%、検査技師で 71.6%、薬剤師で 62.9%の正解率で、これらの 3 職種では比較的高い正解率であったが、看護師、看護学生では 20%代の正解率であり、E 型肝炎という疾患そのものが一般的には知られていない、正しく理解されていないと考えられた。
3. C 型肝炎が食事を介して感染するか否か、針刺し事故での感染確率、蚊を介して感染が成立するかに関する設問では、いずれも医師において正解率が高い結果であった。一方、医師以外の職種、特に看護学生や事務職員では C 型肝炎の感染確率を過大評価していると考えられた。

E. 結論

看護学生 670 名を含む病院職員 5330 名を対象としてウイルス肝炎の感染経路及び感染確率に関する理解度を明らかにする目的で実施した無記名アンケート調査の結果、以下の 3 点を明らかにした。

1. B 型肝炎は、血液を介して感染し空気感染しないということに対する理解度については、国家資格を有する者、医療従事者として患者に直接かかわる職種では、概ね正しく理解されていると考えられた。
2. E 型肝炎という疾患そのものが一般的には知られていない、正しく理解されていないと考えられた。
3. C 型肝炎が食事を介して感染するか否か、針刺し事故での感染確率、蚊を介して感染が成立するかに関する理解は、医師以外の職種では、概ね C 型肝炎の感染確率を過大評価していると考えられた。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

1)　Sawai H, Nishida N, Khor SS, Honda M, Sugiyama M, Baba N, Yamada K, Sawada N, Tsugane S, Koike K, Kondo Y, Yatsuhashi H, Nagaoka S, Taketomi A, Fukai M, Kurosaki M, Izumi N, Kang JH, Murata K, Hino K, Nishina S, Matsumoto A, Tanaka

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2) Izumi N, Takehara T, Chayama K, Yatsuhashi H, Takaguchi K, Ide T, Kurosaki M, Ueno Y, Toyoda H, Kakizaki S, Tanaka Y, Kawakami Y, Enomoto H, Ikeda F, Jiang D, De-Oertel S, McNabb BL, Camus G, Stamm LM, Brainard DM, McHutchison JG, Mochida S, Mizokami M. Sofosbuvir-velpatasvir plus ribavirin in Japanese patients with genotype 1 or 2 hepatitis C who failed direct-acting antivirals. Hepatol Int. 2018 Jul;12(4):356-367.

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2. 学会発表

なし

H. 知的所有権の取得状況（予定を含む）

1．特許取得

該当なし

2．実用新案登録

該当なし

3．その他

該当なし

医療の場における肝炎ウイルス感染予防の事態を知るためのアンケート調査

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共同研究者　四柳　宏　東京大学医科学研究所先端医療研究センター感染症分野　教授

研究要旨

医療の場における肝炎ウイルス感染予防の事態を知るため、日本病院会に加盟している組織に対するアンケート調査を計画した。倫理審査を通過した後アンケートを実施した。来年度に集計・解析を行う予定である。

A. 研究目的

2016 年 10 月から 0 歳児を対象としたＢ型肝炎ワクチン（HB ワクチン）の定期接種が開始され、日本においても今後新規感染は激減することが期待される。しかし多くの人はＢ型肝炎ウイルス（HBV）に対する免疫を有しておらず、こうした人に対する HB ワクチンの接種は今後大切な課題である。

HB ワクチンの接種はハイリスク者（不特定多数の血液・体液に触れる機会の多い人）においては特に大切である。医療従事者はその代表であり、HB ワクチンの接種およびその後の経過観察が非常に大切である。

大きな病院では感染対策チーム（Infection Control）が設けられ、職員の HB ワクチンの接種がきちんと把握されていることが職業感染対策として求められている。しかしながらその実態に関しては施設による格差が大きいと思われる。

今回多施設を対象として HBV 感染対策の実態調査を行った。

B. 研究方法

（図 1）に示すアンケートを作成した。主要調査項目としては医療従事者に対する HB ワクチン接種の実態・接種後の抗体価の把握実態、抗体価低下の際の追加接種の実態などである。

東京大学医科学研究所・東京大学医学部のそれぞれで倫理審査を行った。通過を待って（30-61-B1227）日本病院協会の施設を中心とした約 2000 施設にアンケートを送付した。

C. 研究結果

アンケートの送付を済ませたところであり、これから来年度にかけて結果を解析する予定である。

D. 考察

病床 800 床以上の 92 施設を対象として 2014 年に行われた日本職業感染研究会の調査によれば、稼働 100 床あたり 7 件の針刺しが報告されている。日本全体では 140 万床があることを考えると 10 万件程度の針刺しがあると推定される。現在日本人の HBs 抗原陽性率は約 0.6%であり、年間 600 件程度 HBs 抗原陽性血への曝露が起きていると推定できる。

曝露後の感染は 30%に起きると報告されている。日本ではＢ型肝炎患者の多くが核酸アナログ製剤を飲んでおり、感染率は 30%より低いと思われるがその実態を明らかにすることが大切である。

今回の調査対象施設を含めた多施設で今後ワクチン接種後の感染実態に関する後ろ向き・前向き解析を行うことを計画している。

E. 結論

現在の医療従事者 HBV ワクチン接種率の把握と抗体価推移に関する研究を進める必要性が高い。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

1) Yoshikawa,T, Wada k, Lee JJ, Mitsuda T, Kuroshi H, Aminaka M, Morisawa Y, Morikane K, Kunishima H, Kidouchi K, Moriya K. Changes in twenty years of the epidemiological status of needlestick/sharps injuries reported to japan-epinet through a nation-wide surveillance network. Occup Environ Med 75; suppl2: A341

2. 学会発表等

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H. 知的所有権の取得状況（予定を含む）

1．特許取得
該当なし

2．実用新案登録
該当なし

3．その他
該当なし

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患者や患者の血液・体液に接する可能性のある全ての医療従事者には B 型肝炎ワクチンの接種が行われていますが、ワクチン不応者や抗体低下者に対する対応は医療施設によって異なります。このような状況を鑑み、全国の医療施設を対象に、HB ワクチンの接種状況・感染状況に関するアンケート調査を行うことにしました。アンケート調査結果をもとに、今後の HB ワクチンの接種についての戦略を検討し、また、ワクチン不応者や抗体低下者に対しての対応の標準化を図ることを目指します。

お忙しいところ恐縮ですがご協力のほど宜しくお願い申し上げます。

なお、このアンケートは厚生労働科学研究（肝炎ウイルスの新たな感染防止 - 残された課題・今後の対策 - ）として行うものであり、東京大学大学院医学系研究科・医学部及び東京大学医科学研究所の倫理審査委員会の承認並びに機関の長の許可を得ています。

アンケートへのご回答は任意であり、ご回答及びご返送をもって、同意いただけたものとみなします。アンケートの結果はまとめて報告し、皆様にもご報告しますが、それぞれの施設の現状が公表されることはありません。（ただし、提出された施設を当方のみが把握できるよう、アンケートに通し番号がふってあります。）

【アンケート返送先】
〒108-8639　東京都港区白金台 4-6-1 東京大学医科学研究所　先端医療研究センター感染症分野
四柳　宏

図 1-1　医療従事者への HB ワクチン接種状況に関するアンケート調査のお願い

以下、該当するものに〇を付け、また、適宜（　　）に記入をしてください。

1　あなたの職種をお答えください。
① 医師
② 看護師
③ その他（　　）

2　あなたの施設の規模をお答えください。
① 外来のみ
② 20 床未満
③ 20 以上 100 床未満
④ 100 以上 300 床未満
⑤ 300 以上 500 床未満
⑥ それ以上

3　あなたの施設には専従の ICD がいますか。
① 専従の ICD がいる
② ICD はいるが専従ではない
③ ICD はいない
④ わからない

4　入職時 HB ワクチンの接種が済んでいる職員（あなたの施設で対象となる職種全員に対する割合）の割合はおよそどのくらいですか。
① 20%未満
② 20-39%
③ 40-59%
④ 60-79%
⑤ 80%以上
⑥ 把握していない

図 1-2　アンケート

5　HB ワクチン接種者が抗体陽性になったかをどのように把握していますか。（複数回答可）

① 施設内で採血を行う

② 抗体検査の伝票を提出してもらう

③ 抗体カードに記入してもらう

④ ICT あるいは病院に自己申告してもらう

⑤ その他（　　）

⑥ 把握していない

6　あなたの施設では施設として職員の HBs 抗体獲得状況を把握していますか。（複数回答可）

① データベースファイルを持っている

② 紙にまとめたデータベースを持っている

③ 職員に記録用紙を渡し、自己管理してもらっている

④ その他（　　）

⑤ 把握していない

7　あなたの施設で HB ワクチンの接種対象としている職種を全てお答えください。また対象となる職員数の合計はおおよそ何名ですか。

① 医師

② 看護師

③ 臨床検査技師

④ 放射線技師

⑤ リハビリテーション技師

⑥ 薬剤師

⑦ 事務職員

⑧ その他

対象となる総職員数　　（　　名）

8　あなたの施設で HB ワクチン接種後 HBs 抗体陰性者に対して行っている対応をお答え下さい。（複数回答可）

① CDC の指針に従い、３回の追加接種を行う。

② HBs 抗体陰性の場合は本人の希望があれば追加接種を行う

③ 筋肉内接種を行う

④ 皮内接種を行う

図 1-3　アンケート

⑤　倍量投与を行う
⑥　ワクチンの種類を変更する
⑦　特に対応していない

9　HBs 抗体陽性者に対する再検査を行っていますか。
①　１年に１度行う
②　本人の希望があれば行う
③　特に再検査は行っていない
④　その他（　　）

１０　HBs 抗体陽性となった人に対する追加接種を行っていますか。
①　HBs 抗体が 100mIU/mL 未満になったところで行っている
②　HBs 抗体が 10mIU/mL 未満になったところで行っている
③　行っていない
④　その他（　　）

１１ 今後研究班では、ブースター接種の有無により HBV への感染に差があるかどうかの前向き検討を行う二次調査を予定しています。あなたの施設ではこの検討への参加を希望されますか。
①　希望する
②　条件次第で希望する
③　希望しない

※希望される場合は、別紙１に今後連絡させて頂く場合の連絡先を以下にご記載ください。

１２　その他ご意見があればお書き下さい。

アンケートは以上です。ご協力ありがとうございました。

図 1-4 アンケート

二次調査への参加を希望される場合には、ご担当者の連絡先をお書きください。

お名前

メールアドレス　　@

FAX

図 1-5　アンケート

病院勤務者の肝炎ウイルス感染モニタリングのための全国データベース作成と肝炎ウイルス感染予防状況の実態調査の準備状況

研究分担者 **細野 覚代** 名古屋市立大学大学院医学研究科公衆衛生学分野 研究員

研究要旨

日本では、肝炎ウイルス感染高リスクの医療従事者に対するHBワクチン追加接種の是非についてこれまで十分検討されていなかった。本研究班は、医療従事者や病院勤務者の肝炎ウイルス検査データを収集し、HBワクチン追加接種の効果を検討するために、その基盤となるデータベースを構築する。

パイロット研究として、1996年以降に名古屋市立大学病院に勤務する医療従事者と病院勤務者を対象とするデータベース作成準備を行った。学内の倫理審査の承認が得られ次第、病院内の担当部署から提供された肝炎ウイルス検査データとHBワクチン接種状況に関するデータをリンケージし、データベースを作成する。将来的に他の医療機関のデータセット統合を配慮し、病院独自のIDではなく統合データベース共通IDを作成する予定である。この連結可能匿名化と対応表作成を行うデータ加工プログラムの開発も進めている。

各医療機関でデータ管理状況が異なるため、克服すべき課題もそれぞれ異なる。来年度以降は名古屋市立大学での経験を生かして研究事務局と協力しつつ、他の医療機関のサポート体制について議論を進める予定である。

A. 研究目的

日本環境感染学会のガイドラインでは、B型肝炎(HB)ワクチンを接種し一旦HBs抗体価が陽性（10 mIU/mL以上）と判定された場合の追加接種は必要ないとしている。一方で、HBs抗体価が低下した場合に、B型肝炎ウイルス(HBV)感染の報告が散見されている。本研究班は、肝炎ウイルス感染のハイリスク集団である医療従事者や病院勤務者の肝炎ウイルス検査データを収集し、感染高リスクの医療従事者に対するHBワクチン追加接種の是非を検討するため、基盤となるデータベースを構築する。

肝炎ウイルス感染のハイリスク集団である医療従事者や病院勤務者の肝炎ウイルス検査データとHBワクチン接種状況データを経時的に収集し、肝炎ウイルス感染予防状況の実態調査を行うための全国規模のデータベース作成を目指す。

B. 研究方法

1. データ登録システムの基本構想

名古屋市立大学病院を始めとし、本研究班の研究分担者が所属する医療機関に勤務する医師や看護師、臨床検査技師等の医療従事者と事務職員の肝炎ウイルス検査とHBワクチン接種に関するデータを経時的に収集する。

各医療機関でデータ管理状況は異なるが、図1で示すような流れでデータ登録を進める。まず、各医療機関の責任者が肝炎ウイルス検査・ワクチン接種状況に関するデータをまとめ、①

施設調査データを作成する。ここで、本研究班で独自に開発した②データ加工プログラムを使用して、施設の ID を本研究の共通 ID に変換する。このデータ加工プログラムは、③対応表 (ID 管理ファイル) を作成する機能も備えている。本データ加工プログラムによって、①施設調査データは連結可能匿名化された④事務局提出用データとなり、名古屋市立大学大学院医学研究科ウイルス学分野学内の研究事務局に電子データとして提出される。

研究事務局スタッフは、提出されたデータセットをクリーニングした後、研究事務局内のローカル PC 上で稼働している⑤統合データベースに取り込む。定期的にデータを更新し、新規登録症例数を増やしたり、新たなイベントの把握に務める。

将来、本データベースを活用することで HBV のハイリスク集団である病院勤務者における新たな HBV 感染の有無を確認し、HB ワクチンの長期予防効果を検討することができる。また、HB ワクチン追加接種の状況、HBV 感染の要因 (特に感染経路)、HBV 感染者への対応 (治療の有無など) などの情報を統合し、今後の肝炎ウイルス感染対策に役立てる。

作業効率やデータバックアップも考慮して、将来的に統合データベースをクラウド上で管理する可能性もある。

本年度は、パイロット研究として名古屋市立大学病院に勤務する医療従事者と勤務者の肝炎ウイルス検査と HB ワクチン接種状況に関するデータを収集し、データベース作成を行うことにした。

2. 名古屋市立大学病院におけるパイロット研究

名古屋市立大学病院における研究対象は 1996 年（平成 8 年) 以降に当院に勤務し、肝炎ウイルス検査を実施された 20 歳以上の男女とした。将来の解析のために対象者の職種を、医師・歯科医師、看護職、臨床検査技師、放射線技師、臨床工学士、薬剤師、看護助手・歯科衛生士・歯科技工士、病院職その他、事務、不明に分類した。

図 2 で示すように、名古屋市立大学病院では肝炎ウイルス検査データは中央臨床検査部が管理している。また、HB ワクチン摂取データは医療安全管理室が管理している。

当院の研究審査委員会の承認後、検査部データベース (TOMORROW システム) より 1996 年 − 2018 年 12 月までに実施された肝炎ウイルス検査データを抽出する予定である。肝炎ウイルス検査データセットは、血液検査情報と検診 ID、氏名、性別、生年月日、職種、部門の個人識別情報を含む。

また、HB ワクチン接種状況は医療安全管理室の感染対策業務プログラムを使って 1996 年 − 2018 年 12 月までのデータを抽出する予定である。さらに、感染対策業務プログラムを使って、職員番号・検診 ID・氏名・生年月日・性別・部門・職種情報を含む対応表を作成する。

後日に本研究班活動で測定する HBs 抗原・HBc 抗体価データを検診 ID で肝炎検査ウイルス検査データセットとリンケージする。

これらのデータをとりまとめ、図 1 における①施設調査データを作成する。②データ加工プログラムを用いて、施設調査データの検診 ID を本研究の共通 ID に置き換える。この段階で、氏

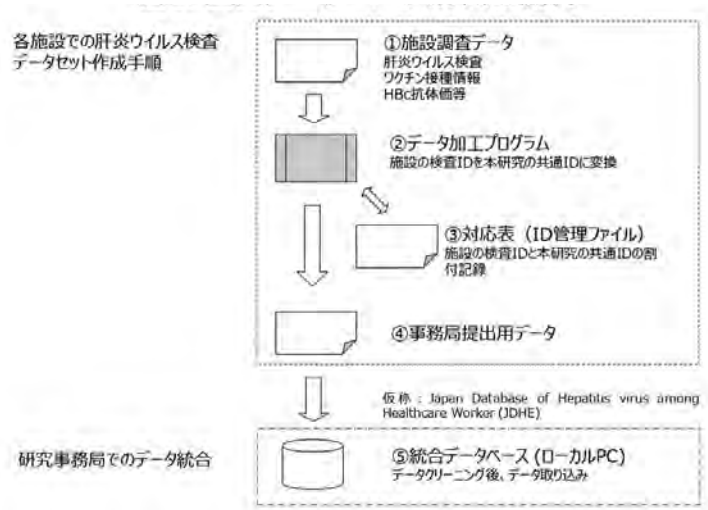

図 1　全国データベース作成の流れ

名は削除し、生年月日データも生年月データに加工し、図１ ④事務局提出用データを作成する。元の検診 ID と共通 ID、氏名、生年月日の対応表を作成し (図１ ③対応表)、将来の調査に備える。

なお、当院で収集する検査項目は以下の通りである。

1) 肝炎ウイルス検査データ：職員番号、検診 ID、氏名、生年月日、年齢、性別、部門、職種、AST、ALT、γ -GTP、HBs 抗原、HBs 抗体価、HCV 抗体価

2) 本研究班で測定した HBs 抗原・HBc 抗体価 (経過観察中に HBs 抗体価が 10 mIU/mL 未満に低下した症例のみ測定予定)

3) HB ワクチン接種状況に関するデータ：職員番号・検査 ID・氏名・生年月日・性別・部門・職種、HB ワクチン接種日 (追加接種がある場合は同様に確認)、HB ワクチン接種歴は予防接種予診票や HB ワクチン接種希望調査の情報も参考にする予定である。

現在、病院内の各部署と調整を行い、学内の倫理審査承認が得られ次第、データセット作成を開始できる状態である。

なお、肝炎ウイルス検査データベース作成とそれを活用する研究に関する説明と同意は、連結可能匿名化した既存情報を使用し、侵襲は無いため、オプトアウトにより研究対象者等が研究参加拒否を表明できる機会を保証する。研究対象者等への告知を名古屋市立大学病院ホームページに掲載する準備も進めている。

3. 統計学的事項

本研究では、名古屋市立大学病院を含む 8 病院 (予定) の勤務者を対象に肝炎ウイルス感染予防状況の実態調査を行うための基盤作成を目的としている。現代の医療行為の現場で肝炎ウイルス感染が成立する可能性は低く、なるべく多数のデータを収集する必要がある。そのため、本研究の目標症例数は 12,000 例、可能な限り多数のデータを収集する。

研究目的にしたがい、収集されたデータを用いて統計解析を実施するが、まず HBs 抗体価低下をアウトカムとしたカプランマイヤー解析とログランク検定を行う予定である。職種別にも検討する。年齢、性別等の交絡因子を調整し、コックス比例ハザードモデルも実施する。

有意水準は P 値 0.05 以下とする。

C. 研究結果・D. 考察

病院勤務者の肝炎ウイルス感染モニタリングのための全国データベース作成準備に取り組んでいる。パイロット研究として、今年度は名古屋市立大学病院のデータベース作成に取り組んだ。

肝炎ウイルス検査や HB ワクチン接種に関する情報統合は本データベースの根幹をなす。しかし、これらのデータは各医療機関で管理が異なるため、克服すべき課題も異なる。可能な限り多数のデータを収集するためには、データ登録を行う医療機関の責任者をサポートする必要があるかもしれない。来年度以降は名古屋市立大学での経験を生かして研究事務局と協力しつつ、他の医療機関のサポート体制について議論

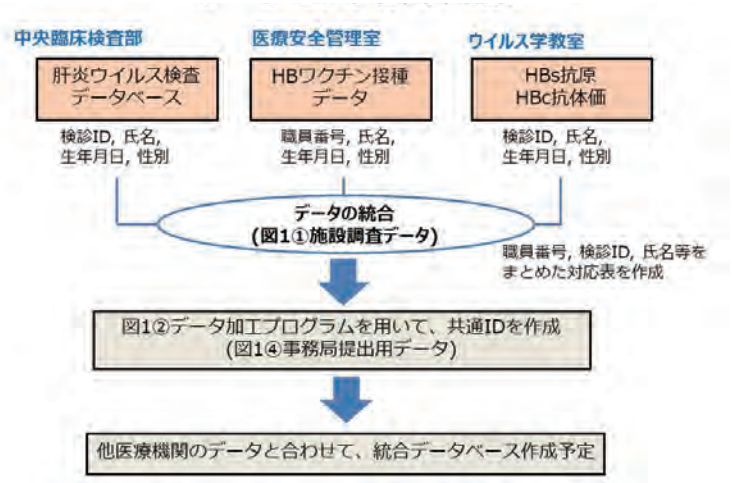

図２ 名古屋市立大学病院のデータ管理状況とデータセット作成の流れ

を進める予定である。

医療従事者の HB ワクチン接種状況と HBV マーカーに関するレジストリーを構築することで、HBs 抗体獲得後のブースター接種の必要性を検討する基礎資料となる。また、青年期以降の HB ワクチン接種効果の検証にも応用可能である。その結果、青年期以降の HB ワクチン接種の必要性に関する基礎資料となることも期待される。

E. 結論

名古屋市立大学病院における肝炎ウイルス検査と HB ワクチン接種状況に関するデータベース作成準備状況を報告した。来年度以降はこの事例を参考として、他の医療機関のサポート体制整備を進めていく。

F. 健康危険情報

なし

G. 研究発表

1．論文発表

なし

2．学会発表

なし

H. 知的所有権の取得状況（予定を含む）

1．特許取得

該当なし

2．実用新案登録

該当なし

3．その他

該当なし

肝炎ウイルスの新たな感染防止・残された課題・今後の対策

研究分担者 田中 靖人 名古屋市立大学大学院医学研究科 教授

研究要旨

B 型肝炎ワクチン（HB ワクチン）定期接種化以前に出生した小児の B 型肝炎感染疫学の調査として、エコチル調査・愛知ユニットセンターに登録された 8 歳学童期調査および 8 歳詳細調査の参加者を対象に HBV 感染の実態調査を行う。対象者数は約 2,500 人であった。また、全国多施設共同研究により医療関係者を対象とした肝炎ウイルス検査データおよび HBV 感染予防状況の実態調査を行い、データベースを構築する。対象は 1996 年以降に研究参加医療機関に所属した 20 歳以上の男女とし、12,000 人を目標とする。今後、できるだけ多くのデータを収集し、肝炎ウイルス感染の有無、HB ワクチン接種により HBs 抗体価が一旦陽性（10 mIU/mL 以上）と判定された者の抗体価の継時的な観察、HBs 抗体価が 10 mIU/mL 未満に低下した者には書面上で同意を得た上で採血を実施し、HBs 抗原・HBc 抗体価を測定する予定である。

A. 研究目的

2016 年 10 月より B 型肝炎ワクチン（HB ワクチン）の 0 歳児定期接種が開始されたが、それ以前の定期接種が実施されていない環境下での HBV 感染の実態は十分に把握できていない。また現在、感染対策としての HB ワクチン接種は、HBs 抗体価が陽性（10 mIU/mL 以上）と判定された時点で免疫獲得とみなし、追加接種は不要とされている。しかしながら HBs 抗体の陽転者を経時的に観察した調査は十分になされていない。本分担研究では、1) 定期接種が開始される前に出生した学童期の小児を対象に HBV 感染の実態を調査する。2) 医療関係者を対象に全国多施設共同研究により検査データを収集し、HBV 感染予防の実態を調査しデータベースを構築する。

B. 研究方法

1) 環境省「子どもの健康と環境に関する全国調査（エコチル調査）」愛知ユニットセンターに登録された児のうち、8 歳学童期調査および 8 歳詳細調査の参加者を対象とする。書面上で同意を得た上で質問票調査、採血を実施し、HBs 抗原･HBc 抗体価を測定する。質問票では、輸血歴、血液製剤の使用歴、HB ワクチンの接種歴、同居家族に「B 型肝炎と診断されている方」がいるかどうかを調査する。

2) 1996 年以降に名古屋市立大学病院および研究参加医療機関に所属し、肝炎ウイルス検査を受けた 20 歳以上の男女のうち、研究参加拒否を表明しなかった者を対象とする。肝炎ウイルス検査データ、HB ワクチン接種歴を収集する。また、経過観察中に HBs 抗体価が 10 mIU/mL 未満に低下した者には書面上で同意を得た上で採血を実施し、HBs 抗原・HBc 抗体価を測定する。いずれか陽性の場合は、詳細な問診による調査を行う。

（倫理面への配慮）

環境省およびエコチル調査コアセンター、名古屋市立大学倫理委員会の審査・承認を得て実施する。新規の採血には必ずインフォームドコンセントを取得し、既存のデータおよび試料も

含めて不同意の機会を担保する。解析データの公表に際しては個人情報保護を徹底する。

C. 研究結果

学童期における検査は、2018 年 10 月時点でエコチル調査 8 歳学童期調査および 8 歳詳細調査の参加者を合わせた約 2,500 人が対象となることを確認した。医療関係者のデータ収集については、2018 年 12 月時点で名古屋市立大学病院の勤務者のうち対象者数は約 6,000 人が見込まれ、さらに参加施設 8 病院の勤務者からできるだけ多くのデータを収集する予定である（目標数 12,000 例）。

D. 考察

B 型肝炎は 1986 年以降の母子感染対策により、垂直感染は激減したが、父子感染を代表とする水平感染が現在も散見される。そのため、定期接種が開始される前に出生した小児の HBV 感染実態を詳細調査することは疫学的な有用性のみならず、ワクチン接種の啓発となることも期待される。

日本環境感染学会の「医療関係者のためのガイドライン」や米国 CDC のガイダンスでは、HB ワクチン接種による HBs 抗体の陽転後、経年により抗体価が低下しても急性肝炎や B 型慢性肝炎の発症予防効果は 20 年以上持続することから、追加接種は不要とされている。しかし、医療関係者は常に感染高リスク環境下に置かれており、HBs 抗体陽転者のモニタリングは追加接種の是非を検討するための重要な資料となる。

E. 結論

HBV 感染疫学、HBs 抗体価の追跡調査を行い、感染と予防の双方から実態の把握を図る。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

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H. 知的所有権の取得状況（予定を含む）

1．特許取得
該当なし

2．実用新案登録
該当なし

3．その他
該当なし

保育の場における肝炎ウイルス感染予防の理解及び実践を図るための保育施設勤務者に対するアンケート調査

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研究要旨

『集団生活の場における肝炎ウイルス感染予防ガイドラインの作成のための研究』（平成 24-26 年度）において作成した保育現場におけるガイドライン（『保育の場において血液を介して感染する病気を防止するためのガイドライン－ウイルス性肝炎の感染予防を中心に－』）の理解度及び感染対策の実際を検証するために、大阪市内の保育施設勤務者にアンケート調査を行った。310 施設、1542 名から回答があった。ガイドラインの認知度は約 2 割で、B 型肝炎が血液から感染することの理解は約 6 割、体液から感染しやすいことの理解は約 2 割、ワクチンで予防できることの理解は約 6 割であった。感染対策に関してはタオル、布団の使用は個別化が進み感染対策されていたが、傷の手当て・軟膏塗布などの血に触れる可能性のある処置における手袋の使用は十分ではなく、今後の啓発が必要である。

A. 研究目的

前研究『集団生活の場における肝炎ウイルス感染予防ガイドラインの作成のための研究』（平成 24–26 年度）において一般生活者･保育関係者･老人施設勤務者を対象とした感染予防のためのガイドラインを作成した。肝炎ウイルスの新たな感染を防ぐためには、これらガイドラインが各現場で活用され、予防策が実施されることが必要である。本研究では保育現場においてガイドライン（『保育の場において血液を介して感染する病気を防止するためのガイドライン－ウイルス性肝炎の感染予防を中心に－』）の感染予防策が浸透しているかを、保育施設勤務者へのアンケート調査から検証を行った。

B. 研究方法

大阪市ホームページに掲載されている大阪市内の保育施設 694 施設に対し、2018 年 12 月から 2019 年 2 月に郵送にてアンケート調査を行った。アンケートの内容は、①保育施設勤務者のガイドラインの認知度及び B 型肝炎ワクチン接種率、②保育施設勤務者のウイルス肝炎の理解度、③保育施設での感染対策の現状、④入所（園）児のワクチン接種の把握と保育施設勤務者によるワクチン接種指導について行った。各施設に 5 枚のアンケート用紙を送付し、アンケート調査に賛同の職員が多い施設はアンケート用紙をコピーして返答していただいた。この研究は大阪急性期・総合医療センターの倫理委員会の承認を得て行った。

C. 研究結果

（1）アンケート回答数と回答施設・職種

310 施設（回答率 45％）、1542 名から回答があった。施設規模では園児 1-20 名の施設 27％、21–45 名 4％、46–60 名 6％、61-90 名 19％、91–120 名 20％、121–150 名 14％、151 名以上 10％であった。職種では施設長 15％、保育士 73％、

看護師 5%、事務員 2%、その他（調理師・栄養士・保育補助員など）5%であった。看護師の勤務している保育施設は全体の 38%、看護師の巡回がある施設は 6%あった。

(2) 保育施設勤務者のガイドラインの認知度

保育施設勤務者の 19%が保育の場におけるガイドラインを知っていた。看護師では 43%、保育士では 16%が知っていた。

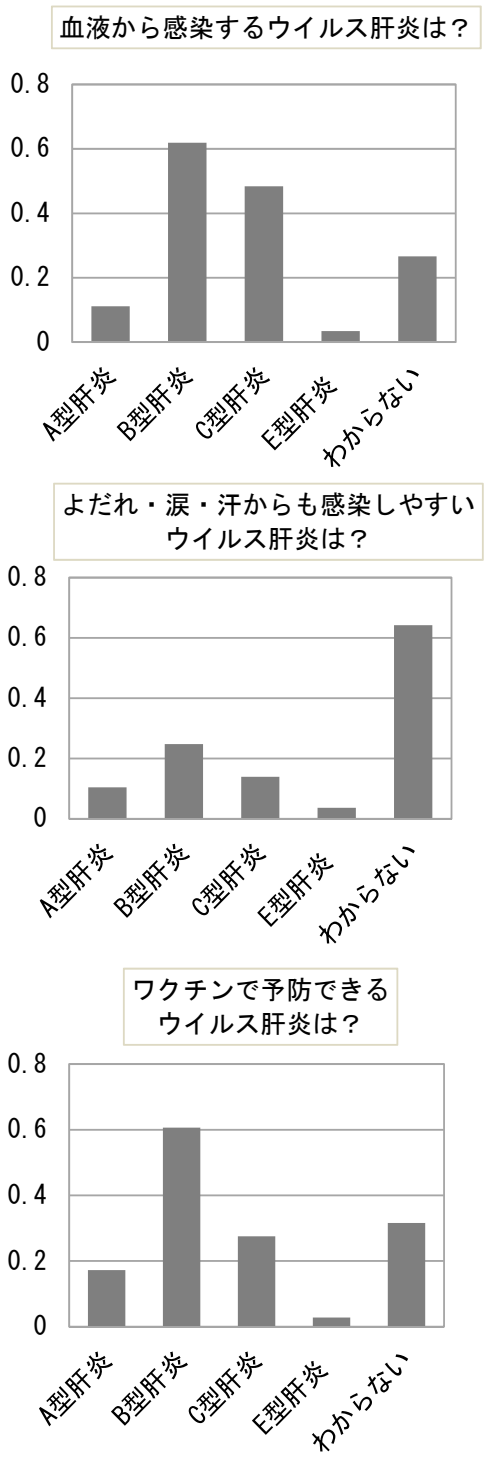

図 1

(3) 保育施設勤務者の B 型ワクチン接種率と B 型肝炎キャリア

保育施設勤務者の 10%が B 型肝炎ワクチンを接種していた。看護師では 74%、保育士では 7%の接種率であり、子どもに最も接触する保育士の B 型肝炎ワクチン接種率は高くはなかった。また、10 名（0.66%）が B 型肝炎キャリア、3 名（0.2%）が B 型肝炎既感染であった。

(4) 保育施設勤務者のウイルス肝炎認知度（図 1）

保育施設勤務者の 99%が A 型、B 型、C 型、E 型のいずれかのウイルス肝炎を聞いたことがあった。B 型肝炎は 92%、C 型肝炎は 84%が聞いたことがあった。

「血液を介して感染するウイルス肝炎はどれか」の設問に対しては、B 型肝炎を 62%、C 型肝炎を 48%が回答していた。しかし、B 型肝炎と C 型肝炎の 2 つを回答したのは 28%であった。

「よだれ・涙・汗からも感染しやすいウイルス肝炎はどれか」の設問に対しては、わからないという回答が 64%と最も多く、B 型肝炎のみを回答したのは 14%であった。

「ワクチンで予防できるウイルス肝炎はどれか」の設問に対しては、B 型肝炎と回答したのは 61%であったが、わからないという回答も 32%と次に多かった。A 型肝炎と B 型肝炎の 2 つを回答したのは 9%であった。

(5) 保育施設での感染対策の現状（図 2）

排便のあるおむつ交換時の手袋着用は 81%で「必ず」されていたが、傷の手当てでは 17%、軟膏の塗布では 38%であった。

手洗いタオルの使用に関しては、96%がタオルを園児間で共有しない、もしくは使い捨てペーパータオルを使用していた。布団の使用に関しても 88%が共有していなかった。

(6) 入所（園）児のワクチン接種の把握と保育施設勤務者によるワクチン接種の指導

入所（園）児のワクチン接種の保育施設勤務者による把握は 75%の施設で「必ず」行われていた。そして、接種漏れに気が付いた場合、保育施設勤務者の 36%は「必ず」、25%は「だいたい」、ワクチン接種をするように指導していた。

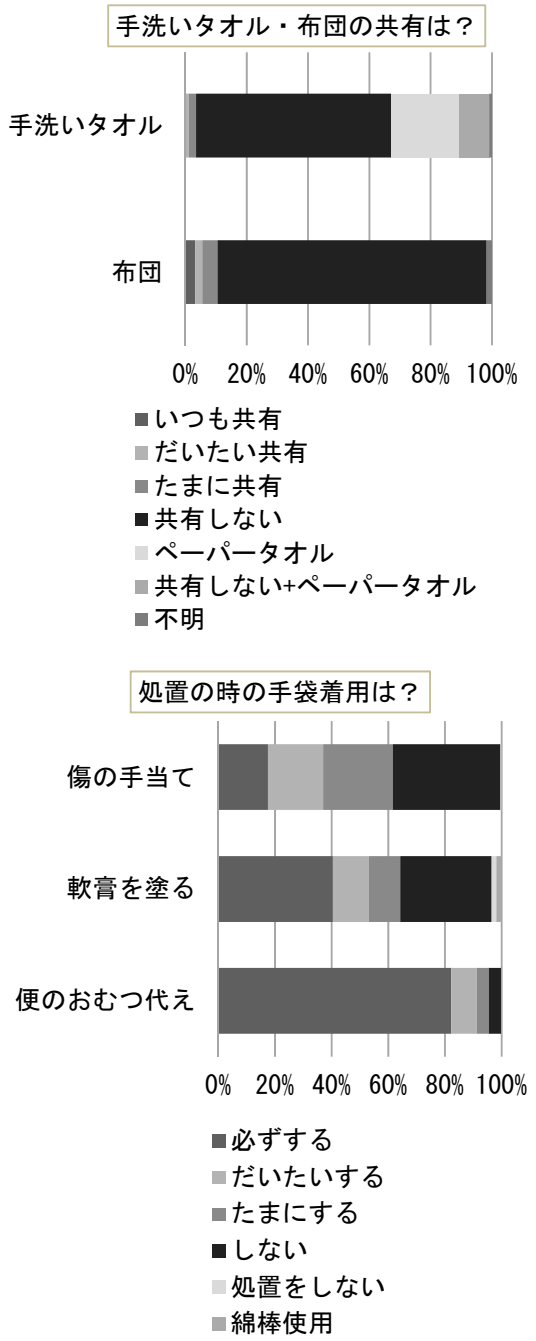

図２

D. 考察

保育施設勤務者の 19％が保育の場におけるガイドラインを知っており、特に看護師での認知度が高かった。保育施設の 44％が看護師の勤務もしくは巡回があり、看護師を中心に感染対策が行われている保育施設が多いと考える。保育施設の感染対策の中心となる職員への教育を行い、ガイドラインの理解度を深めるのがよいと考える。

保育施設勤務者のウイルス肝炎の理解度に関して、B 型肝炎、C 型肝炎が血液感染であることは約半数が回答したが、確実に理解しているのは約 3 割と言える。B 型肝炎が体液から感染しやすいことの理解は不十分である。各ウイルス肝炎においてどのような場合に感染しやすいか、感染経路の啓発が必要である。また、ワクチン接種が B 型肝炎の予防に有効であることも理解しているのは約 6 割であり、十分ではない。

感染予防対策に関しては、タオルの使用、布団の使用は個別化が進んで共有が少なく、感染対策がされている。さらに排便の処理に関しても手袋の使用が多い。しかし、傷の手当てや軟膏塗布における手袋使用は十分ではなく、啓発が必要である。

最後に、保育施設勤務者が入所（園）児のワクチン接種について 75％が把握しており、保育施設勤務者からワクチン接種漏れの指導がさらに行われるようになると有効でないかと考える。

E. 結論

保育施設勤務者におけるウイルス肝炎感染予防ガイドラインの認知度は約 2 割で、ウイルス肝炎の感染経路に関する理解も十分ではない。タオル、布団の使用は個別化が進み感染対策されているが、傷の手当て・軟膏塗布などの血に触れる可能性のある処置における手袋の使用は十分ではなく、今後の啓発が必要である。

F. 健康危険情報

総括研究報告書にまとめて記入する。

G. 研究発表

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3) 高野 智子, 田尻 仁, 虻川 大樹, 乾 あやの, 惠谷 ゆり, 鈴木 光幸, 水落 建輝, 三善 陽子, 村上 潤：小児期 B 型肝炎水平感染例の診断年齢による臨床的特徴の違い：第 45 回日本小児栄養消化器肝臓学会（2018/10/6　さいたま）
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H. 知的所有権の取得状況（予定を含む）

1．特許取得
該当なし
2．実用新案登録
該当なし
3．その他
該当なし

小児における B 型肝炎ワクチン定期接種後の疫学調査

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研究要旨

小児における B 型肝炎ウイルスの感染実態および B 型肝炎ワクチン定期接種開始後のワクチン接種率・HBs 抗体獲得率・HBs 抗体持続期間を明らかにするため、病院受診者の残余検体を用いた多施設共同疫学調査を開始した。

A. 研究目的

2016 年 10 月からすべての乳児を対象とする B 型肝炎（HB）ワクチンの定期接種が開始された。

定期接種開始前の小児における HBs 抗原陽性率は 0.03％程度と極めて低かったが、HBc 抗体陽性率は 0.5–1％と想定以上に高く、小児においても水平感染が起こっている可能性が示唆された。一方で、定期接種開始前の HB ワクチン接種率は極めて低く、10 歳以上では約 1–2％であった。

定期接種開始後 2 年が経過したが、開始後の HBV 感染実態やワクチン接種率の詳細は不明であり、定期接種の効果は明らかとなっていない。

そこで本研究では、B 型肝炎ワクチン定期接種開始後のワクチン接種率・HBs 抗体獲得率・HBs 抗体持続期間および小児における B 型肝炎ウイルスの感染実態を明らかにすることを目的とした。

B. 研究方法

協力病院を受診し、採血検査をうけた 0 ～ 15 歳の小児の残余検体を用いて統一した測定方法で HBs 抗体および HBc 抗体を測定する。母子手帳から生年月日、性別、HB ワクチン接種回数および最終 HB ワクチン接種年月日を確認する。

（倫理面への配慮）

小児を対象とした研究であり、侵襲的な行為が加わらないように残余検体を用いる。公開文書あるいは個別同意書を用いて保護者の同意を得る。

＜主要評価項目＞

・HBc 抗体陰性かつ HBs 抗体陽性率を HB ワクチンによる抗体陽性率とする。

・HBc 抗体陽性率を HBV 感染率とする（HBs 抗体の＋／－は問わない）。

＜副次評価項目＞

母子手帳から HB ワクチン接種率が明らかになる。ワクチン接種者中の HBs 抗体陽性率から HB ワクチン有効率が推定できる。年齢ごとの HBs 抗体保有率と HBV 感染率を比較検討する。

残余検体に余りがあれば、HBV 感染者の詳細な状態（HBs 抗原、HBV-DNA、HBV-genotype など）を明らかにする。

C. 研究結果

本年度は、多施設共同疫学調査の計画を作成し、各協力病院での倫理審査および同一の検査方法での測定ができるように体制作りを行った（図 1）。

特に、HBs 抗体は測定キットにより測定値が異なること、HBc 抗体は定期接種開始前の疫学調査と比較検討を可能にするために、統一した測定方法（ルミパルス HBs 抗体、ルミパルス HBc 抗体）を用いることとして、検査体制を決定した。

筑波大学附属病院を代表施設として、倫理委員会の承認を得た。神戸こども急性期医療センター、日本大学附属板橋病院で承認済、筑波メディカルセンター病院、茨城県立こども病院、神戸こども急性期医療センターなどで承認予定であり、来年度以降、検体収集及び解析開始予定である。

D. 考察

倫理委員会承認申請および検査体制が整った。

E. 結論

来年度以降、検体検査を進める予定である。

F. 健康危険情報

なし

G. 研究発表

1．論文発表

なし

2．学会発表

なし

H. 知的所有権の取得状況（予定を含む）

1．特許取得

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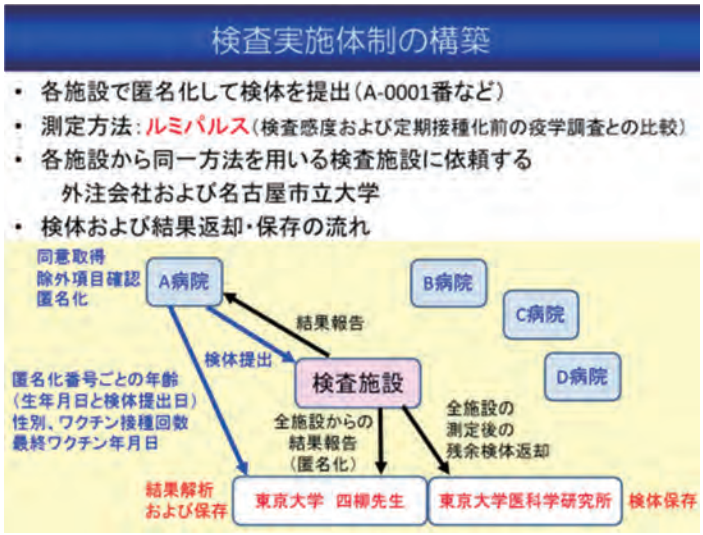

図 1

B 型肝炎ワクチン定期接種化後の本邦小児における B 型肝炎ウイルス感染およびワクチン接種の実態調査

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研究要旨

本研究では、定期接種開始後の B 型肝炎（HBV）ワクチン接種率、および有効率、抗体持続率とともに、HBV 感染率を評価し、今後の HBV 感染予防策に有用な知見を得る事を目的としている。本年度は、筑波大学を主研究機関としたグループ（筑波メディカルセンター病院、茨城県立こども病院、総合守谷第一病院、日本大学医学部附属板橋病院、神戸こども初期急病センター、大阪急性期・総合医療センター、静岡厚生病院、東京大学医学部附属病院）を結成した。そして、2019 年度からの本研究の遂行に向けて、日本大学医学部附属板橋病院および神戸こども初期急病センターの倫理委員会の承認を得て、研究体制を整えた。

A. 研究目的

2013-2015 年度、厚生労働科学研究費補助金による研究班（研究代表者：筑波大学医学医療系小児科　須磨崎亮）により、本邦小児における B 型肝炎（HBV）感染を明らかにするための疫学調査が行われた。HBs 抗原陽性率は約 0.03％と想定通り低かったものの、HBs 抗原陰性・HBc 抗体陽性率が 0.5-1.0％と想定以上に高く、健常小児においても B 型肝炎の水平感染が散発していることが推測され、2016 年 10 月からすべての乳児を対象として B 型肝炎ワクチンの定期接種が開始された。本研究では、定期接種開始後の HB ワクチン接種率、および有効率、抗体持続率とともに、HBV 感染率を評価し、今後の HBV 感染予防策に有用な知見を得る事を目的とした。

B. 研究方法

被験者の選定方針：

日本大学医学部附属板橋病院および神戸こども初期急病センターを受診した小児患者および保護者に対し、公開文書（病院の採血場所およびホームページに掲示）を用いて説明を行い、1 ヶ月以内に不同意の申し出がなかった人を対象とする。検体収集時に、疾患名から、免疫不全や輸血歴など特殊なリスクをもつことが推測される患者を除外する。

方法：

検体および臨床情報（年齢、性別、既往歴）を収集する。①１ヶ月間不同意の申し出がないことを確認し、検査部保管の検体をピックアップする、②臨床情報収集（電子カルテから、年齢、性別、疾患名を収集し、匿名化番号と対応するよう符号表を作る）、③重複検体（過去に検体としてピックアップした同一人物の検体）ではな

いことを確認する。

検体と臨床情報は、連結不可能匿名化して、筑波大学研究担当者に報告された後、対象者となる検体につき、外注会社（どの協力施設からも統一された会社に依頼し、測定方法を統一する）に依頼し、HBs 抗体、HBc 抗体の測定を行い、ワクチンによる抗体陽性率、HBV 感染率についての統計学的解析を行う。また、診療録、母子手帳の記載および問診（保護者の記憶等）からB 型肝炎ワクチン接種率を明らかにする。B 型肝炎ワクチンによる抗体陽性率、HBV 感染率、接種率から、ワクチン有効率を明らかにし、最終ワクチン接種後○年時の抗体陽性率から、HBs 抗体自然減衰が明らかとなり、ハイリスク者への追加接種の議論における基礎データとする。

（倫理面への配慮）

本研究では、日本大学医学部附属板橋病院および神戸こども初期急病センターで、診療目的で採血され、研究目的に保護者から書面にて使用の同意を得られている残余検体を用いて行うものである。本研究のために、改めて同意をとることはきわめて困難である。そこで、同意については、日本臨床検査医学会の指針に基づき、「同意を得ることが困難な場合は試料が連結不可能匿名化されている場合、あるいは当該研究が公衆衛生の向上のために特に必要であって、当該研究に関する試料等の利用目的を含む情報の公開、被検者による拒否の機会の確保という条件を満たす場合に倫理委員会の承認と施設長の許可を得て研究を実施することができる」と記されており、本研究はこれに沿って行う。不同意の場合、公開文書に不同意の場合の連絡先を記載し、申し出てもらうことで意思確認をする。

また、感染症というデリケートな項目を測定するため、上記のとおり残余検体については、連結可能匿名化し、研究開始時には連結不可能匿名化を行う。結果については、被験者および保護者、診療医、研究者のいずれも個人とリンクした形の情報はもちえない。したがって、被験者および保護者、主治医からの問い合わせにも対応はできない。

C. 研究結果

筑波大学を主研究機関としたグループ（筑波メディカルセンター病院、茨城県立こども病院、総合守谷第一病院、日本大学医学部附属板橋病院、神戸こども初期急病センター、大阪急性期・総合医療センター、静岡厚生病院、東京大学医学部附属病院）を結成した。そして、2019 年度からの本研究の遂行に向けて、日本大学医学部附属板橋病院および神戸こども初期急病センターの倫理委員会の承認を得た（日本大学医学部附属板橋病院：2019 年 2 月 12 日、神戸こども初期急病センター：2019 年 2 月 28 日）。また、小児科内での本研究内容の周知を行い、診療科をあげての研究体制を整えた。

D. 考察

本研究により、定期接種開始後の HB ワクチン接種率、乳児における HB ワクチン接種の有効率、HBs 抗体持続期間、HBc 抗体陽性率が明らかとなる。これにより、被験者を含む定期接種の効果（感染率低下、抗体保有率上昇）を評価するのみならず、乳児における HB ワクチンの有効率や抗体持続期間などを検討することで、今後の被験者を含む国民の B 型肝炎ウイルス感染症の制御対策に有用な知見が得られることが期待できる。

E. 結論

2019 年度からの本研究の遂行に向けて、日本大学医学部附属板橋病院および神戸こども初期急病センターの倫理委員会の承認を得、研究の準備が整った。

F. 健康危険情報

なし

G. 研究発表

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研究会、研修会

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H. 知的所有権の取得状況（予定を含む）

1．特許取得
該当なし
2．実用新案登録
該当なし
3．その他
該当なし

本年の急性肝炎の疫学に関する動向

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研究要旨

急性肝炎に関する疫学情報は少ない。本研究では、感染症法を基に感染症サーベイランス事業で届け出された急性肝炎症例について報告する。特に、本年は A 型急性肝炎のアウトブレイクが見られたので、感染症サーベイランス事業の結果と定点医療施設の観察結果と比較する。さらに、A 型急性肝炎のアウトブレイクは米国でも見られたので、その状況と対策を参考にするため解析した（本研究は感染研疫学センターと共同で行われた）。

A. 研究目的

急性肝炎の発生動向の把握は、1987 年に感染症サーベイランス事業の対象に加えられ、全国約 500 カ所の定点病院からの調査として開始された。その後、1999 年 4 月の感染症法施行により、四類感染症の「急性ウイルス性肝炎」として全数把握疾患となり、さらに 2003 年 11 月の感染症法の改正に伴い四類（A, E 型肝炎）、五類感染症の（B, C 型肝炎等）に分類され、その発生動向が監視されている。本研究では、感染症法のもとで、診断・報告された急性肝炎について報告する。さらに、定点医療機関での観察結果、海外でのアウトブレイクの状況とその対策を含めて報告する。

B. 研究方法

(1) 定点医療施設における A 型急性肝炎の観察

2012 年より、東京都新宿区の HIV 陽性男性同性愛者が多い医療施設で急性肝炎の定点観察を行っている。定点医療施設において見出された急性 A 型肝炎の遺伝子レベルでの解析を行った。

(2) 感染症サーベイランス事業による A 型急性肝炎の疫学

急性肝炎に関する疫学情報は少ない。本邦での感染症法に基づく感染症サーベイランスは感染源の発生や流行を探知することができ、蔓延を防ぐための対策や医療従事者、国民への情報提供に役立っている。本研究では届け出された急性肝炎症例の年別発生状況、年齢別分布、都道府県別報告状況、感染経路等について解析した。

(3) 米国における A 型急性肝炎の動向と対策

本邦と同様な先進国である米国における A 型急性肝炎のアウトブレイクが見られたので、その状況と対策を解析した。

（倫理面への配慮）

情報については匿名化し、研究班では個人情報を保持しない。また、情報公開の際も個人を識別できる情報は排除する。

C. 研究結果

(1) 定点医療施設における急性肝炎の観察

定点医療施設における A 型急性肝炎は 2012 年から 2017 年まで 1 人も見られなかった。しかし、2018 年 1 月に初めて 2 人が見出された。そこで患者血清サンプルの回収を始めた。1, 2 月は各 2 人、3, 4 月は各 3 人、5 月に 6 人とピークに達した後、6 月 3 人、7 月 1 人、8 月 0 人と終息した。1 月から注意喚起をするとともにワクチン

接種を推奨した。10 人の患者について HAV の塩基配列を比較したところ、全ての症例で完全一致し、さらにこの配列は台湾の流行株と一致した。

(2) 感染症サーベイランス事業による急性肝炎の疫学

急性 A 型肝炎は 2012 年から 2017 年までは、全国的な流行が見られた 2014 年（433 例）を除き、年間約 100 ～ 300 例で推移していたが、2018 年は年はじめから急激な増加を認めた。2018 年は 2015 ～ 2017 年に比べて、都市部の 20–30 代の男性の性的接触が多く、特に男性同性間性的接触の報告数が多かった。

(3) 米国における A 型急性肝炎の動向と対策

米国カルフォルニア州サンディエゴ郡では 2013 年 3 月より A 型急性肝炎の増加を認め、5 月からは毎月 100 人の新規発症が見られた。患者の大多数は、ホームレスおよび違法薬物使用者であった。サンディエゴ郡の HAV 感染者は、年齢およびベースにある健康状態、特に慢性肝疾患のため死亡数が 20 人にのぼった。サンディエゴ郡保健当局は 3 月から月 4000 回のワクチン接種を開始し、発生数の減少が見られなかったため、緊急事態を宣言し 8 月から 2 ヶ月間月 40000 回の接種を行ったところ、やっと発生の減少傾向を認め、翌年初めに終息宣言することができた。この間、ワクチン接種数：194,038 症例（2018 年 8 月 1 日時点）、この地域の HAV 感染に対する郡の費用は、2018 年 1 月までに 970 万ドル、2018 年 4 月末に約 1250 万ドルを要した。米国では、Kentuky, West virginia, Michigan, Ohio 等でもアウトブレイクが続いている。

D. 考察

本年初頭より急性 A 型肝炎のアウトブレイクを認めた。男性同性間性的接触の報告数が多かったことから、男性同性愛者における啓発、ワクチン接種の推奨が重要と考えられた。アウトブレイクは定点医療施設における観察の方が感染症サーベイランス事業より早く検知可能であったとともに、定点医療施設では血清サンプルを用いる解析が可能であった。

米国では急性 A 型肝炎は 80 年代は年間 30000 件も報告されていたが、衛生状況の改善等により 2014 年には年間 1500 人程度まで減少していた。しかし 2017 年ごろから全米各地でアウトブレイクが見られるようになり、米国のような先進国でも急性 A 型肝炎が再興することを示し、一度発生すると沈静化までに莫大なコストがかかることを示した。

E. 結論

急性肝炎の発生動向の全数把握は予防対策、啓発活動に大変有効であると考えられた。また、定点医療機関でのサンプルの遺伝子解析を組み合わせることでより早く、詳細な疫学情報の把握が可能になると期待される。さらに、米国のような先進国でも A 型肝炎が再興することから、抗体保有率の把握とともに日頃からの注意深い情報取集が必要ということがわかった。

F. 健康危険情報

なし

G. 研究発表

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H. 知的所有権の取得状況（予定を含む）

1．特許取得
該当なし
2．実用新案登録
該当なし
3．その他
該当なし

医療ビッグデータを用いた急性肝炎の疫学調査に関する研究

研究分担者　田倉 智之　東京大学大学院医学系研究科医療経済政策学　特任教授

研究要旨

“新たな感染を防ぐ”視点からの疫学調査が肝炎対策の一環で望まれている。本年度は、予備的報告として、医療ビッグデータを応用し、C型肝炎を対象に抽出・連結を行い、試行的にサーベイを実施した。C型肝炎受療群の同居のサンプル数は、患者ベースで 74 人（全体 4,608 人、1.61%）となった。医療ビッグデータを当該領域の疫学調査に応用することについて、幾つかの制約要件も明らかとなったが、有用な手法であることも示唆された。

A. 研究目的

本邦における昨今の肝疾患領域の罹患実態や治療技術の動向を背景に、“新たな感染を防ぐ”視点からの疫学調査が望まれている。

本研究は、(1) 急性肝炎（B型・C型に加えA型）の実態（罹患、地域、時期など）を整理、および (2) C型肝炎の新規発症例の感染経路として家族内伝播のコホート検討を目的とする。

B. 研究方法

本年度は、予備的報告として、医療ビッグデータを応用し、対象群の抽出・連結を行い、試行的にサーベイを実施した。利用したデータソースは、東京大学が管理する医療経済系ビッグデータ（TheBD;約 600 万件× 6 年間）を選択した（医科、調剤）。なお、調査の論点として、以下の内容が挙げられた。

（1）急性肝炎の疫学

「急性期」の定義とデータセットの仕様をどうするか

⇒　前治療歴、治療内容、治療期間、
治療転帰などから関連のマスタ等を作成中

（2）感染経路の疫学

「家族内」の定義および同定、バイアスをどうするか。

⇒　被保険者番号と受療医療機関、受診時期と患者年齢、前治療歴、主副病名（登録時期）、医療機関紹介、等の情報の組み合わせから、対象のコホートを生成中

上記の論点を踏まえ、以下の整理の手法（研究デザイン）を設定し、準備を始めた。

・層別解析：疾病（ICD10）、性・年齢、診療行為（検査・投薬・療養・指導等）、地域等

図 1　データ抽出とデータセット、およびデータ分析の構成

患者数ベース

C型肝炎患者数	同一世帯	同居	非同居
4, 608	74	46	28

【参考】世帯数ベース

C型肝炎患者所属世帯数	同一世帯	同居	非同居
4, 571	37	23	14

図２　C型肝炎罹患における家族内感染の可能性の予備結果

・補正処理:人口動態（エリア含)、季節変動（月次)、施設分布（可能な場合のみ）

・推計分析:マルコフモデル（又は決定木分析)、モンテカルロシミュレーション

横断調査で短期間（2013 年 –2017 年）のサンプルデータ（急性期・慢性期のC型肝炎群）を抽出し、感染経路の疫学の調査等が可能か、ＦＳを実施した。

（倫理面への配慮）

特になし。

C. 研究結果

継続治療群（肝炎関連の薬物療法）として 38,468 人、受診歴有群（検査等）として 70,395 人のサンプルがあった。

うちC型肝炎受療群の同居のサンプル数は、患者ベースで 74 人（全体 4,608 人、1.61%)、世帯ベースで 37 件（全体 4,571 件）となった。

D. 考察

得られた結果は、他の統計情報等と比較しその妥当性の検証が必要と考えられた。以上を踏まえ、今後の研究は、次の内容を予定する。

・サンプリングの精査（新規発症者等）
・急性期と慢性期の精査と層別化実施
・受療医療機関の要件等をより精細化
・治療内容等を考慮した母集団の設定

E. 結論

医療ビッグデータ応用したC型肝炎の感染経路の疫学調査を試行したところ、家族内感染の可能性が示された。

医療ビッグデータを当該領域の疫学調査に応用することについて、幾つかの制約要件も明らかとなったが、有用なアプローチであることも示唆された。

F. 健康危険情報

なし

G. 研究発表

1．論文発表

なし

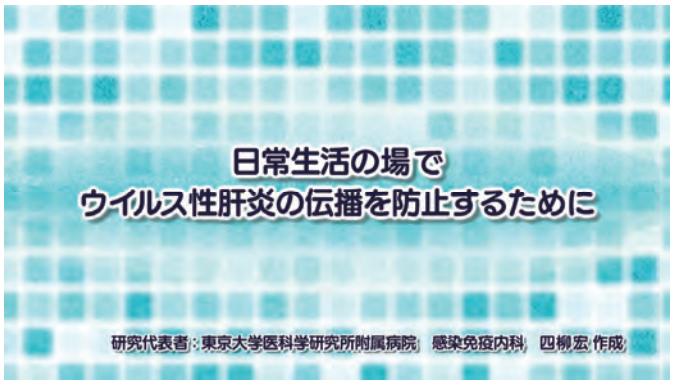
2．学会発表

なし

H. 知的所有権の取得状況（予定を含む）

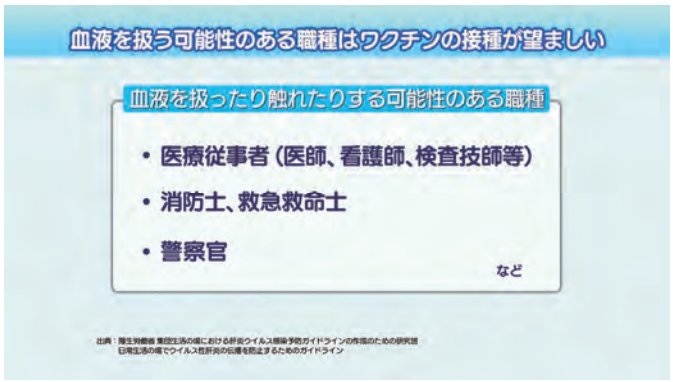
1．特許取得
該当なし
2．実用新案登録
該当なし
3．その他
該当なし

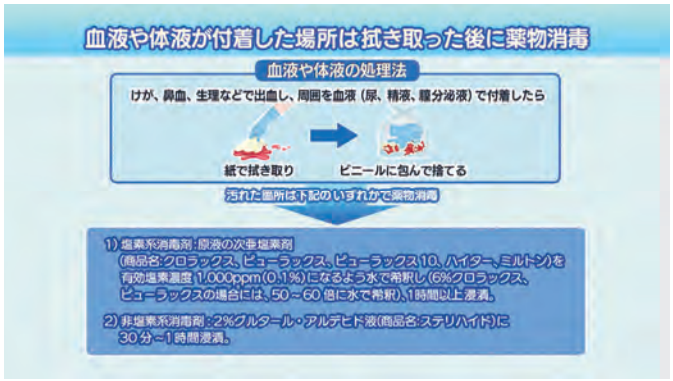
研究成果の刊行物・別刷

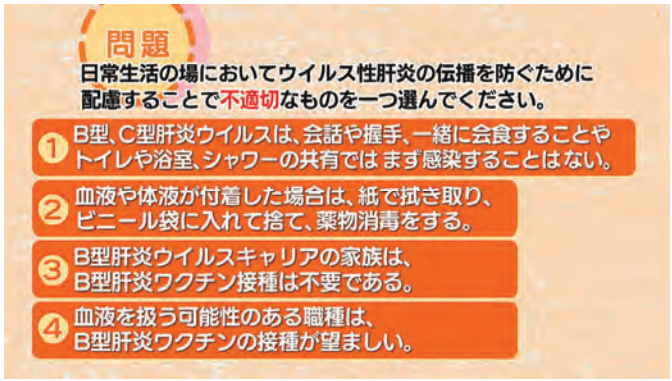

日常生活の場で
ウイルス性肝炎の伝播を防止するために
研究代表者：東京大学医科学研究所附属病院　感染免疫内科　四柳宏 作成


全ての赤ちゃんがB型肝炎ワクチンを接種することが望ましい
2016年10月1日から
B型肝炎ワクチンが定期接種化
• ワクチンを接種することで、
体の中にB型肝炎ウイルスへの抵抗力(免疫)ができます。
• 免疫ができることで、一過性の肝炎を予防できるだけでなく、
キャリアになることを予防でき、まわりの人への感染も防ぐことができます。
※ 予防接種を受けても、お子さんの体質によって免疫ができないことがあります
B型肝炎ワクチンの副作用には
注射した部位の赤み 腫れ しこり 接種後の倦怠感 頭痛
などがあり、ほとんどが無処置で数日中によくなります。

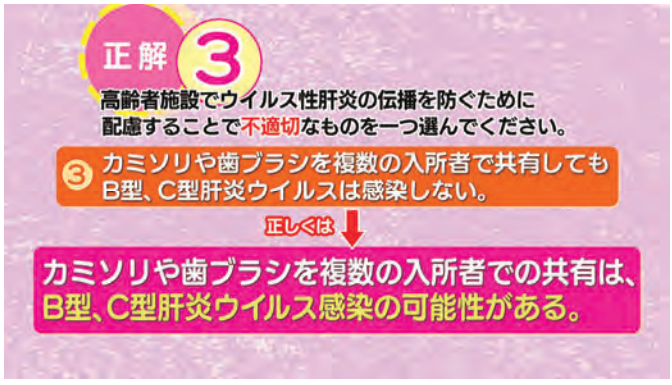

B型，C型肝炎ウイルスは血液や体液を介して感染する可能性がある
肝炎ウイルスに感染する可能性のある行為
肝炎ウイルスに感染する可能性のない行為
血液・体液を介して


血液を扱う可能性のある職種はワクチンの接種が望ましい
血液を扱ったり触れたりする可能性のある職種
• 医療従事者（医師、看護師、検査技師等）
• 消防士、救急救命士
• 警察官
など


血液や体液が付着した場所は拭き取った後に薬物消毒
血液や体液の処理法
けが、鼻血、生理などで出血し、周囲を血液（尿、精液、膣分泌液）で付着したら
紙で拭き取り
ビニールに包んで捨てる
汚れた箇所は下記のいずれかで薬物消毒
1）塩素系消毒剤：原液の次亜塩素剤
（商品名：クロラックス、ピューラックス、ピューラックス10、ハイター、ミルトン）を
有効塩素濃度1,000ppm（0.1%）になるよう水で希釈し（6%クロラックス、
ピューラックスの場合には、50～60倍に水で希釈）、1時間以上浸漬。
2）非塩素系消毒剤：2%グルタール・アルデヒド液（商品名：ステリハイド）に
30分～1時間浸漬。


問題
日常生活の場においてウイルス性肝炎の伝播を防ぐために
配慮することで不適切なものを一つ選んでください。
① B型、C型肝炎ウイルスは、会話や握手、一緒に会食することや
トイレや浴室、シャワーの共有ではまず感染することはない。
② 血液や体液が付着した場合は、紙で拭き取り、
ビニール袋に入れて捨て、薬物消毒をする。
③ B型肝炎ウイルスキャリアの家族は、
B型肝炎ワクチン接種は不要である。
④ 血液を扱う可能性のある職種は、
B型肝炎ワクチンの接種が望ましい。


B型肝炎ウイルスキャリアの家族はワクチンの接種が望ましい
肝炎ウイルス検査で
陰性を確認
ワクチン
家族
B型肝炎ワクチンは3回接種
初回
2回目
3回目
1カ月後
6カ月後
• 感染予防機能を獲得した場合
15年間は効果が持続
• 投与後に免疫を獲得したか
血液検査で確認


正解 3
高齢者施設でウイルス性肝炎の伝播を防ぐために
配慮することで不適切なものを一つ選んでください。
③ カミソリや歯ブラシを複数の入所者で共有しても
B型、C型肝炎ウイルスは感染しない。
正しくは
カミソリや歯ブラシを複数の入所者での共有は、
B型、C型肝炎ウイルス感染の可能性がある。


高齢者施設で
ウイルス性肝炎の伝播を防止するために
研究代表者：東京大学医科学研究所附属病院　感染免疫内科　四柳宏 作成


高齢者施設の職員はワクチンの接種が望ましい
職員
B型肝炎ウイルス
入所者
肝炎ウイルス検査で陰性を確認
ワクチン
B型肝炎ワクチンは3回接種
初回
2回目
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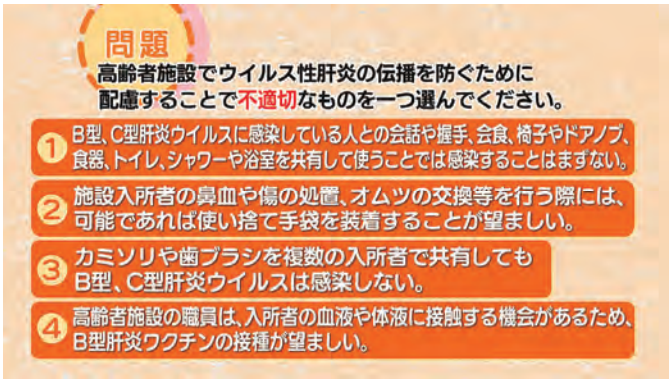
3回目
6カ月後
感染予防機能を獲得した場合15年間は効果が持続
投与後に免疫を獲得したか血液検査で確認


B型，C型肝炎ウイルスは血液や体液を介して感染する可能性がある
肝炎ウイルスに感染する可能性のある行為
肝炎ウイルスに感染する可能性のない行為
血液・体液を介して
性交渉
ピアス
ひげ剃り
風呂
トイレ
椅子・ドアノブ・床
公衆トイレ
筆記用具
食器
シャワー・浴室


肝炎キャリアの職員も高齢者施設での仕事は可能
職員
B型肝炎ウイルス
入所者
血液や体液の処理法
けが、鼻血、生理などで出血し、周囲を血液（尿、精液、腟分泌液）で汚したら
紙で拭き取り
ビニールに包んで捨てる
入所者の皮膚に傷や皮膚炎などがある場合には、入所者への感染を防ぐため自身の血液・体液が触れないよう注意を払う。


施設の日常生活でB型，C型肝炎ウイルスはうつりにくい
肝炎ウイルスに感染する可能性のない行為
風呂やトイレ
食器の共有等で
肝炎ウイルスが
伝播することはまず無い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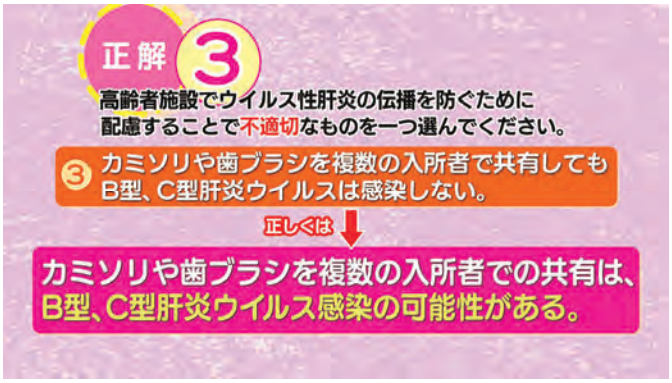
配慮することで不適切なものを一つ選んでください。
① B型、C型肝炎ウイルスに感染している人との会話や握手、会食、椅子やドアノブ、食器、トイレ、シャワーや浴室を共有して使うことでは感染することはまずない。
② 施設入所者の鼻血や傷の処置、オムツの交換等を行う際には、可能であれば使い捨て手袋を装着することが望ましい。
③ カミソリや歯ブラシを複数の入所者で共有しても B型、C型肝炎ウイルスは感染しない。
④ 高齢者施設の職員は、入所者の血液や体液に接触する機会があるため、B型肝炎ワクチンの接種が望ましい。


感染者（入所者，職員）の血液・体液が非感染者（入所者，職員）の皮膚や粘膜の傷から侵入した場合に感染が起こりうる
肝炎ウイルスに感染する可能性のある行為
血液・体液を介して
傷や穴は絆創膏やガーゼで覆い接触感染の危険性を減らしましょう。
医療器具やかみそり、歯ブラシ、ピアッサーなどを他人と共有することは避けましょう。
鼻血や傷の処置、オムツ交換等を行う際には、可能であれば使い捨て手袋を装着しましょう。


正解 3
高齢者施設でウイルス性肝炎の伝播を防ぐために
配慮することで不適切なものを一つ選んでください。
③ カミソリや歯ブラシを複数の入所者で共有しても B型、C型肝炎ウイルスは感染しない。
正しくは
カミソリや歯ブラシを複数の入所者での共有は、B型、C型肝炎ウイルス感染の可能性がある。

保育の場で
ウイルス性肝炎の伝播を防止するために

研究代表者：東京大学医科学研究所附属病院　感染免疫内科　四柳宏 作成

唾液のつくものの扱い方（2）

- 哺乳瓶、乳首、歯ブラシ、コップ同様
  園児の使う寝具、パジャマ、タオルにも唾液がつきます。
- 園児の使った寝具、パジャマ、タオルは使い回しをせず、
  洗濯後 よく乾かしてから使います。
- 肝炎ウイルスに感染している園児の唾液がついたものは、
  50~60倍 希釈の塩素系漂白剤
  （ピューラックス®、ハイター®、ブリーチ®など）に
  10分程度つけてから洗浄し乾燥させます。

B型肝炎ウイルス・C型肝炎ウイルスへの感染は
血液や体液を介して起こります

肝炎ウイルスにはA型からE型まで５つのウイルスがあります。
このうち血液や体液を介して伝播する（うつる）のは
B型肝炎ウイルスとC型肝炎ウイルスです。

B型肝炎

- ✓感染力がC型に比べ強い。
- ✓血液中のウイルス量の多い場合体液の中にウイルスが存在する。
- ✓感染していても症状はないため、誰が感染しているかわからない。

などの問題があり、このことを理解して対応する必要があります。

B型肝炎・C型肝炎を予防するには血液・体液に注意することが基本です。
B型肝炎の予防にはワクチンが効果的です。

傷の手当てについて

- 保育士は自分の手についた傷をばんそうこうなどで
  きちんと覆っておく必要があります。
- これは保育士自身を守るだけではなく、
  保育士から園児への感染を防ぐためでもあります。
- 傷の手当ては
  できれば使い捨て手袋をして行うことが望まれます。

ウイルスは体についた傷から入ります

- B型肝炎ウイルス、C型肝炎ウイルスへの感染は
  体の表面についた傷を通じておこります。
- ころんだ時の傷、ひっかかれた時の傷、
  噛み付かれた時の傷からウイルスが侵入します。
- 指先のささくれ、やけどした皮膚などからも
  ウイルスは侵入します。
- こうした傷をしっかり覆い、
  血液や体液に触れないようにすることが大切です。

B型，C型肝炎ウイルスは血液や体液を介して感染する可能性がある

出典：厚生労働省 集団生活の場における肝炎ウイルス感染予防ガイドラインの作成のための研究班
「日常生活の場でウイルス性肝炎の伝播を防止するためのガイドライン」

唾液のつくものの扱い方（1）

- 唾液の中には肝炎ウイルスだけではなく、
  口の中にある細菌をはじめ いろいろな微生物が
  入っている可能性があります。
- したがって哺乳瓶、乳首、歯ブラシ、コップなどは
  個人専用にするのが原則です。
- 唾液のついたおもちゃなどは
  水洗いしてよく乾かすことが基本です。

全ての赤ちゃんがB型肝炎ワクチンを接種することが望ましい

2016年10月1日から
B型肝炎ワクチンが定期接種化

- ワクチンを接種することで、
  体の中にB型肝炎ウイルスへの抵抗力（免疫）ができます。
- 免疫ができることで、一過性の肝炎を予防できるだけでなく、
  キャリアになることを予防でき、まわりの人への感染も防ぐことができます。

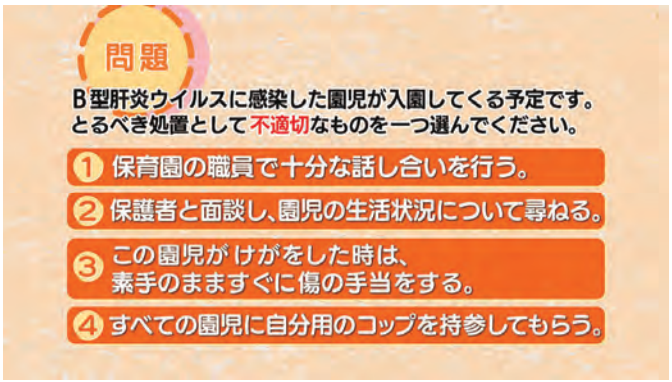
※ 予防接種を受けても、お子さんの体質によって免疫ができないことがあります

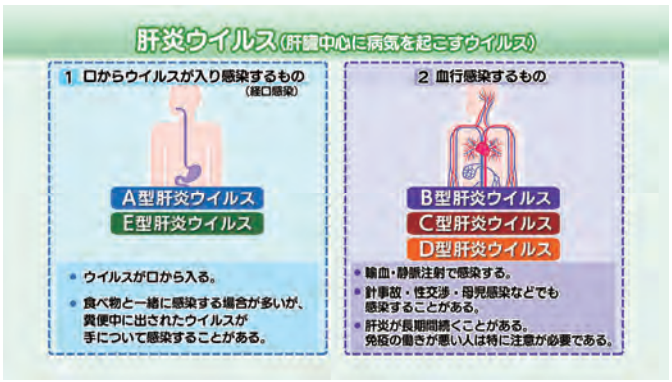
B型肝炎ワクチンの副作用には
注射した部位の赤み　腫れ　しこり　接種後の倦怠感　頭痛
などがあり、ほとんどが無処置で数日中によくなります。

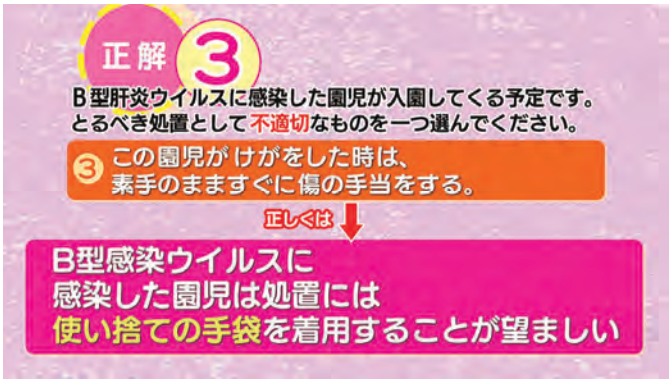
出典：厚生労働省 集団生活の場における肝炎ウイルス感染予防ガイドラインの作成のための研究班
「日常生活の場でウイルス性肝炎の伝播を防止するためのガイドライン」

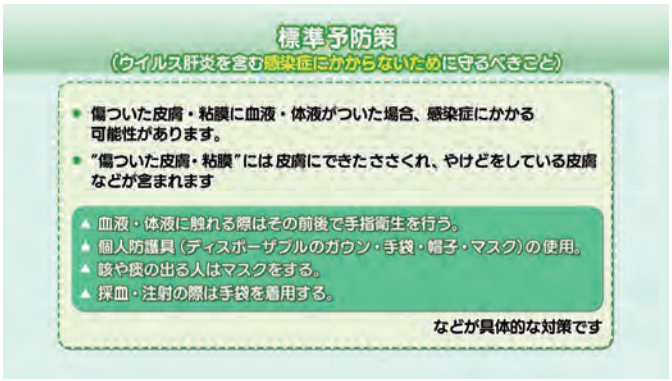

保育士はワクチンの接種が望ましい
職員
園児
B型肝炎ウイルス
肝炎ウイルス検査で陰性を確認
ワクチン
B型肝炎ワクチンは3回接種
初回
2回目
3回目
1カ月後
6カ月後
• 感染予防機能を獲得した場合15年間は効果が持続
• 投与後に免疫を獲得したか血液検査で確認

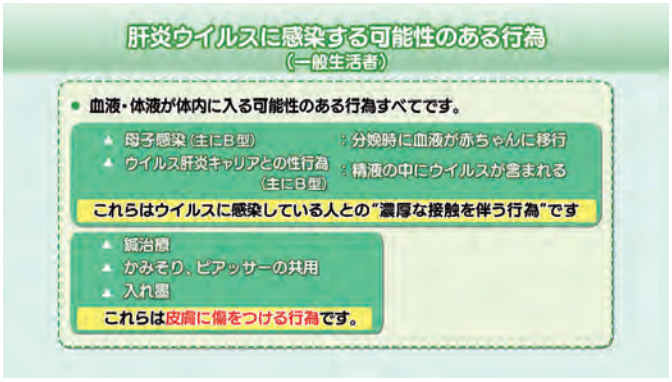

肝炎ウイルスの感染経路について
研究代表者：東京大学医科学研究所附属病院　感染免疫内科　四柳宏 作成

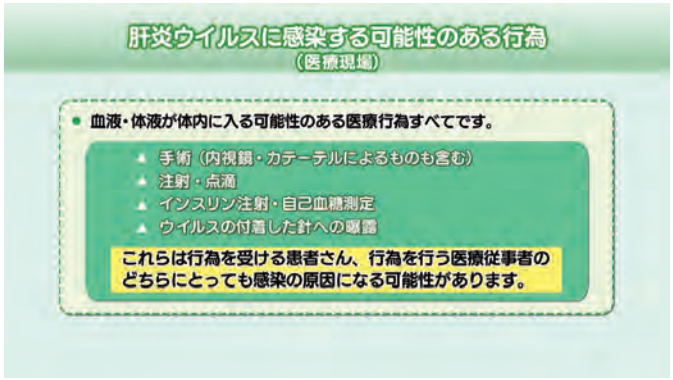

問題
B型肝炎ウイルスに感染した園児が入園してくる予定です。
とるべき処置として不適切なものを一つ選んでください。
① 保育園の職員で十分な話し合いを行う。
② 保護者と面談し、園児の生活状況について尋ねる。
③ この園児がけがをした時は、素手のまますぐに傷の手当をする。
④ すべての園児に自分用のコップを持参してもらう。

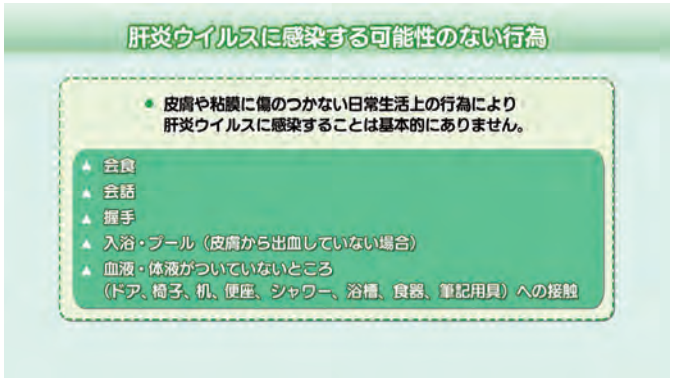

肝炎ウイルス（肝臓中心に病気を起こすウイルス）
1 口からウイルスが入り感染するもの
（経口感染）
A型肝炎ウイルス
E型肝炎ウイルス
• ウイルスが口から入る。
• 食べ物と一緒に感染する場合が多いが、糞便中に出されたウイルスが手について感染することがある。
2 血行感染するもの
B型肝炎ウイルス
C型肝炎ウイルス
D型肝炎ウイルス
• 輸血・静脈注射で感染する。
• 針事故・性交渉・母児感染などでも感染することがある。
• 肝炎が長期間続くことがある。免疫の働きが悪い人は特に注意が必要である。


正解 3
B型肝炎ウイルスに感染した園児が入園してくる予定です。
とるべき処置として不適切なものを一つ選んでください。
③ この園児がけがをした時は、素手のまますぐに傷の手当をする。
正しくは
B型感染ウイルスに
感染した園児は処置には
使い捨ての手袋を着用することが望ましい

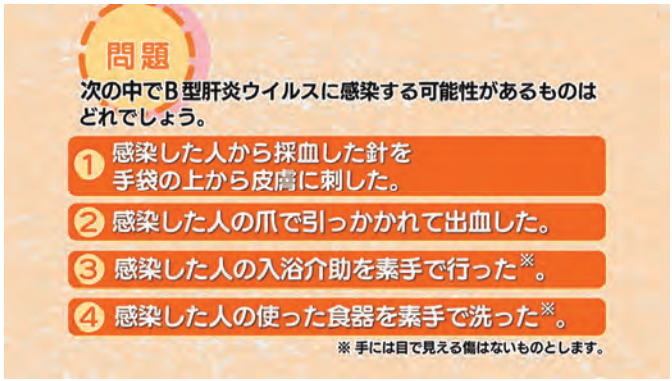

標準予防策
（ウイルス肝炎を含む感染症にかからないために守るべきこと）
• 傷ついた皮膚・粘膜に血液・体液がついた場合、感染症にかかる可能性があります。
• "傷ついた皮膚・粘膜"には 皮膚にできたささくれ、やけどをしている皮膚などが含まれます
▲ 血液・体液に触れる際はその前後で手指衛生を行う。
▲ 個人防護具（ディスポーザブルのガウン・手袋・帽子・マスク）の使用。
▲ 咳や痰の出る人はマスクをする。
▲ 採血・注射の際は手袋を着用する。
などが具体的な対策です

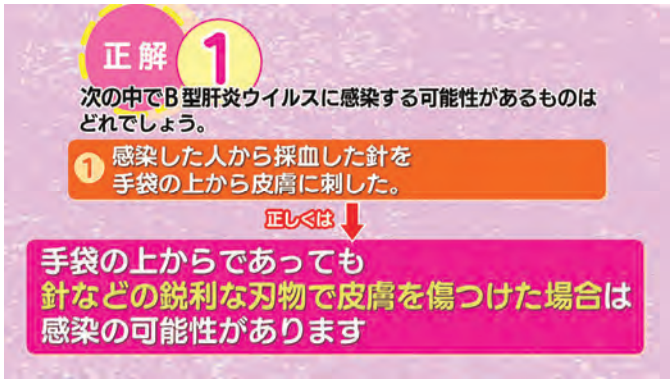

肝炎ウイルスに感染する可能性のある行為
（一般生活者）
• 血液・体液が体内に入る可能性のある行為すべてです。
▲ 母子感染（主にB型）：分娩時に血液が赤ちゃんに移行
▲ ウイルス肝炎キャリアとの性行為（主にB型）：精液の中にウイルスが含まれる
これらはウイルスに感染している人との"濃厚な接触を伴う行為"です
▲ 鍼治療
▲ かみそり、ピアッサーの共用
▲ 入れ墨
これらは皮膚に傷をつける行為です。


肝炎ウイルスに感染する可能性のある行為
（医療現場）
血液・体液が体内に入る可能性のある医療行為すべてです。
手術（内視鏡・カテーテルによるものも含む）
注射・点滴
インスリン注射・自己血糖測定
ウイルスの付着した針への曝露
これらは行為を受ける患者さん、行為を行う医療従事者の
どちらにとっても感染の原因になる可能性があります。


肝炎ウイルスに感染する可能性のない行為
皮膚や粘膜に傷のつかない日常生活上の行為により
肝炎ウイルスに感染することは基本的にありません。
会食
会話
握手
入浴・プール（皮膚から出血していない場合）
血液・体液がついていないところ
（ドア、椅子、机、便座、シャワー、浴槽、食器、筆記用具）への接触


リスクのある行為とない行為をはっきりさせました
肝炎ウイルスに感染する可能性の
ある行為
肝炎ウイルスに感染する可能性の
ない行為
「濃厚な接触」「血液が付着している可能性のあるものの共用」が危険です


問題
次の中でB型肝炎ウイルスに感染する可能性があるものは
どれでしょう。
① 感染した人から採血した針を
手袋の上から皮膚に刺した。
② 感染した人の爪で引っかかれて出血した。
③ 感染した人の入浴介助を素手で行った※。
④ 感染した人の使った食器を素手で洗った※。
※ 手には目で見える傷はないものとします。


正解 1
次の中でB型肝炎ウイルスに感染する可能性があるものは
どれでしょう。
① 感染した人から採血した針を
手袋の上から皮膚に刺した。
正しくは
手袋の上からであっても
針などの鋭利な刃物で皮膚を傷つけた場合は
感染の可能性があります

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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相崎英樹	C 型肝炎ウイルスの遺伝子構造と抗ウイルス薬の作用機序		日本医事新報	日本医事新報社	東京都	2018	64-65

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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ORIGINAL ARTICLE

Sofosbuvir–velpatasvir plus ribavirin in Japanese patients with genotype 1 or 2 hepatitis C who failed direct-acting antivirals

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Abstract

Background/purpose In Japan, there is a growing population of patients with chronic hepatitis C virus (HCV) infection who failed a direct-acting antiviral (DAA)-based regimen. In this Phase 3 study, we evaluated sofosbuvir–velpatasvir plus ribavirin in Japanese patients with genotype 1 or 2 HCV infection who previously received DAAs.

Methods Patients were randomized 1:1 to receive sofosbuvir–velpatasvir plus ribavirin for 12 or 24 weeks. Randomization was stratified by HCV genotype and presence of cirrhosis. The primary endpoint was sustained virologic response 12-week post-treatment (SVR12).

Results Of 117 participants, 81% had HCV genotype 1 infection, 33% had cirrhosis, and 95% had NS5A resistance-associated substitutions (RAS) at baseline. Overall, SVR12 rates were 97% (58/60; 95% CI 88–100%) with 24 weeks of treatment and 82% (47/57; 95% CI 70–91%) with 12 weeks. For HCV genotype 1 and 2 infected patients, the SVR12 rates with 24 weeks of treatment were 98% and 92%, respectively. In both treatment groups, SVR12 rates in HCV genotype 1 patients were statistically superior to a historical control rate of 50% ($p < 0.001$). For patients with NS5A RASs at baseline, 85% (46/54) in the 12-week group and 96% (54/56) in the 24-week group achieved SVR12. The most common adverse events were upper respiratory tract viral infection, anemia, and headache. Three (2.6%) patients discontinued treatment because of adverse events.

Conclusion Sofosbuvir–velpatasvir plus ribavirin was highly effective and well tolerated in Japanese patients who previously failed a DAA-based regimen. Baseline NS5A RASs did not affect treatment outcomes.

Keywords DAA-experienced · NS5B polymerase inhibitor · NS5A inhibitor · Antiviral resistance · Salvage therapy

Abbreviations

ASV	Asunaprevir
BMI	Body mass index
DAA	Direct-acting antiviral
DCV	Daclatasvir
ELB	Elbasvir

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GLE	Glecaprevir
GRZ	Grazoprevir
GT	Genotype
HCV	Hepatitis C virus
LDV	Ledipasvir
LLOQ	Lower limit of quantification
OMB	Ombitasvir
PAR	Paritaprevir
PIB	Pibrentasvir
RAS	Resistance-associated substitution
RBV	Ribavirin
SMV	Simeprevir
SOF	Sofosbuvir
SVR	Sustained virological response
TVR	Telaprevir
ULN	Upper limit of normal
VAN	Vaniprevir
VEL	Velpatasvir

Introduction

In Japan, there is a growing population of patients with chronic hepatitis C virus (HCV) infection who did not achieve sustained virologic response (SVR) with a direct-acting antiviral (DAA) regimen. The standard of care in Japan for chronic HCV infection has been evolving since the first DAA agent, telaprevir, was approved in 2011 for use in combination with peginterferon-alfa and ribavirin. In 2014, the all-oral regimen of daclatasvir, HCV NS5A inhibitor, and asunaprevir, HCV NS3/4A protease inhibitor, was approved for patients with chronic HCV genotype 1 infection [1]. Although the combination provided an interferon- and ribavirin-free treatment option, its overall efficacy has been suboptimal compared to newer DAA-based regimens. In a study of 222 Japanese patients with HCV genotype 1b, 15% experienced virologic failure with daclatasvir plus asunaprevir [2]. Failure rates were higher (59%) in patients with baseline NS5A resistance-associated substitutions (RASs), and treatment failure was associated with the emergence of RASs in the gene sequences for both NS5A and NS3/4. Separate analyses have evaluated the RAS profiles of patients who failed treatment with daclatasvir and asunaprevir. In one study, 63% of patients had dual NS5A RASs at L31 and Y93 at the time of failure [3]. A second study demonstrated that 91% had RASs at the time of virologic failure, including 52% with 2 RASs, 27% with 3 RASs, and 6% with deletions at NS5A sites 29 or 32 [4].

At the time this study was initiated, Japanese patients with HCV genotype 1 who had failed daclatasvir plus asunaprevir had very limited and complicated treatment options. The 2017 Japanese Society for Hepatology guidelines for hepatitis C treatment recommended that daclatasvir plus asunaprevir failures who were eligible to receive interferon be retreated with the NS3/4A inhibitor simeprevir plus peginterferon and ribavirin [5]. Those who were intolerant to or ineligible for interferon were recommended to receive ledipasvir–sofosbuvir as long as they did not have multiple resistance mutations in the NS5A region. For patients who did have multiple NS5A resistance mutations, who comprise the majority of daclatasvir plus asunaprevir failures [3, 4], a "wait-and-see" approach was recommended. Such patients had limited retreatment options, and they were typically excluded from clinical trials of novel HCV drugs.

The combination of sofosbuvir, NS5B polymerase inhibitor, with velpatasvir, NS5A inhibitor, is a once-daily, oral, pan-genotypic single-tablet regimen that is well tolerated and leads to high SVR rates (95–99%) in patients with or without compensated cirrhosis [6, 7]. Combining sofosbuvir–velpatasvir with ribavirin has the potential to be a salvage regimen for Japanese patients who have failed a DAA-containing regimen. In a previous Phase 2 study of patients who were DAA-experienced, treatment with sofosbuvir–velpatasvir plus ribavirin for 24 weeks resulted in SVR12 rates of 97% in patients with HCV genotype 1 and 93% in those with HCV genotype 2 [8]. In this Phase 3 study, we evaluated the efficacy and safety of sofosbuvir–velpatasvir plus ribavirin for 12 or 24 weeks in Japanese patients with genotype 1 HCV infection who were previously treated with NS5A inhibitor or genotype 2 HCV infection with any DAA-containing regimen.

Methods

Patients

Patients $\geq$ 20 years old with plasma HCV RNA $\geq 10^4$ IU/mL and chronic genotype 1 or 2 HCV infection that had previously not achieved SVR with a DAA-containing regimen lasting at least 4 weeks were eligible to enroll. For patients with HCV genotype 1, the DAA regimen must have included NS5A inhibitor. Patients without cirrhosis or with compensated cirrhosis were eligible for participation; the presence of cirrhosis was determined by either (1) liver biopsy with Metavir 4 or Ishak $\geq$ 5 scores; (2) Fibroscan $>$ 12.5 kPa; or (3) FibroTest score $\geq$ 0.75. Key exclusion criteria included noncompliance with the most recent DAA-containing regimen, previous discontinuation of sofosbuvir and ribavirin because of intolerance, body

weight < 40 kg, platelets < 50,000/μL, hemoglobin < 10 g/dL, alanine aminotransferase or aspartate aminotransferase > 10 × upper limit of normal (ULN); direct bilirubin > 1.5 × ULN; hemoglobin A1c > 8.5%; creatinine clearance (Cockcroft–Gault) < 50 mL/min; albumin < 3 g/dL; International Normalized Ratio of prothrombin time > 1.5 × ULN; infection with hepatitis B or HIV; or porphyria.

Study design

This was a Phase 3, multicenter, open-label study. Via an interactive web response system, patients were randomly assigned 1:1 to 12 or 24 weeks of treatment with sofosbuvir–velpatasvir (400 mg/100 mg) fixed-dose combination tablet once-daily and weight-based ribavirin (REBETOL®, MSD KK) 600–1000 mg divided twice daily. Randomization was stratified by cirrhosis status (presence or absence) and HCV genotype (1 or 2). Approximately 90 patients with HCV genotype 1 and 20 patients with HCV genotype 2 were targeted for enrollment. Across the study population, approximately 20 were to have compensated cirrhosis. After completing 12 or 24 weeks of treatment, all patients underwent follow-up visits at post-treatment weeks 4, 12, and 24.

Study oversight

The study protocol was approved by the review board or ethics committee of each institution prior to study initiation. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines and the Declaration of Helsinki. Patients provided written informed consent before undertaking any study-related procedures.

Assessments

Screening assessments included measurement of plasma HCV RNA level, HCV genotyping, *IL28B* genotyping, and standard laboratory and clinical tests. HCV RNA levels were quantified using the COBAS Ampliprep/COBAS TaqMan HCV Test, v2.0 (Roche Molecular Systems, Inc., Branchburg, NJ), which has a lower limit of quantitation (LLOQ) of 15 IU/mL. HCV genotype and subtype was determined using the Siemens VERSANT® HCV Genotype INNO-LiPA2.0 Assay. *IL28B* genotype was determined by polymerase chain reaction amplification of the single-nucleotide polymorphism rs12979860, with sequence-specific forward and reverse primers and allele-specific fluorescently labeled TaqMan® minor groove binder probes.

Plasma HCV RNA levels were evaluated at screening; on day 1 of treatment; at treatment weeks 1, 2, 3, 4, 5, 6, 8, 10, and 12 for all patients and weeks 16, 20, and 24 for those receiving 24 weeks of treatment; and at post-treatment weeks 4, 12, and 24. Missing SVR values were imputed as a success if bracketed by values that were termed successes.

Plasma samples for viral sequencing were collected at all treatment and follow-up visits, following the same schedule as for HCV RNA evaluation. RASs present in more than 15% of the sequence reads are reported. Deep sequencing of the NS5A and NS5B coding regions was performed on samples obtained from all patients at baseline and from those with virologic failure at the time of failure.

Safety assessments included physical examinations and vital sign assessments conducted at all study visits. In addition, adverse events and concomitant medication intake were ascertained and clinical laboratory assessments were collected at screening, every treatment visit, and at the post-treatment week 4 visit.

Endpoints

The primary efficacy endpoint was achievement of SVR12, defined as having HCV RNA < LLOQ 12 weeks after discontinuing study drugs. The primary safety endpoint was discontinuation of study drugs due to adverse events.

Statistical analyses

Because of the limited number of patients with HCV genotype 2 patients in this study, the sample size justification was based on genotype 1 patients only. A sample size of 45 HCV genotype 1 patients in each treatment group was to provide over 90% power for the primary efficacy analysis, which was to detect at least 27% improvement in SVR12 rate from a historical control rate of 50% using a two-sided exact one-sample binomial test at significance level of 0.025 with Bonferroni alpha adjustment. The 50% SVR null rate was derived from SVR rates of 43% (59/137) and 59% (57/96) (116/233 = 50%) for treatment-naive patients with genotype 1 HCV infection and high viral loads treated with peginterferon and ribavirin for 48 weeks cited in the Japanese package inserts for REBETOL® Capsules 200 mg (MSD, July 2015, 19th version) and COPEGUS® Tablets 200 mg (Chugai Pharmaceuticals, July 2015, 6th version), respectively. No statistical hypothesis testing was performed for the groups of patients with HCV genotype 2. A point estimate with two-sided 95% exact confidence interval using the binomial distribution (Clopper–Pearson method) was constructed for the SVR12 rates in each treatment group. Also explored in post hoc analyses were factors associated with treatment

failure. Exact logistic regressions were conducted using the relapse rate in 3 groups: all patients, patients infected with genotype 1 in both treatment groups combined, or patients treated for 12 weeks. Analysis variables were selected based on the size of the population and potential for impacting treatment success. The factors analyzed included sex, age group (< 65 or ≥ 65 years), absence or presence of cirrhosis, baseline HCV RNA (< 5 log10 IU/mL or ≥ 5 log10 IU/mL), number of RAVs (< 2 or ≥ 2), absence or presence of the NS5A RAVs L31 in combination withY93, adherence rate (< 80% or ≥ 80%), treatment duration (12 or 24 weeks), and RBV dosage as a continuous variable measured by number of tablets taken.

Results

Patient population

From August of 2016 through March of 2017, 117 patients were treated at 18 study sites in Japan. The median age for the study population was 64 years (range 21–81) (Table 1). Thirty-three percent (39/117) of patients had cirrhosis. Fifty-seven percent (67/117) had a non-CC *IL-28B* genotype. Among patients with genotype 1 infection, 97% (92/95) had subtype 1b. Most patients (84%, 83/117) had undergone 2 or more prior DAA treatment regimens. The median (range) reported duration of the most recent prior DAA treatment was 14 (7–36) weeks in the 12-week group and 12 (6–36) in the 24-week group. Seventy-five percent (88/117) of patients were previously treated with both NS5A and NS3/4 inhibitors, including 8 patients who had also been treated with NS5B inhibitor. Among patients with genotype 1 HCV infection, the most common prior treatment regimen was daclatasvir plus asunaprevir (86%, 82/95), and, among patients with genotype 2 HCV infection, the most common prior DAA was sofosbuvir (91%, 20/22). Ninety-five percent of patients (110/116) had 1 or more NS5A RASs at baseline, including 71% (82/116) with 2 or more NS5A RASs. Of the 117 patients who were enrolled, 114 (97%) completed treatment (Fig. 1).

Efficacy

Overall, SVR12 rates were higher with 24 weeks versus 12 weeks of treatment (Table 2). In the 12- and 24-week treatment groups, 82% (47/57; 95% CI 70–91%) and 97% (58/60; 95% CI 88–100%) of patients achieved SVR12, respectively. Among patients with HCV genotype 1, SVR12 rates were 85% (40/47; 95% CI 72–94%) with 12 weeks and 98% (47/48; 95% CI 89–100%) with 24 weeks. The SVR12 rates of sofosbuvir–velpatasvir plus ribavirin for 12 weeks ($p < 0.001$) and 24 weeks ($p < 0.001$) in HCV genotype 1 patients were both statistically superior to the historical control rate of 50%. For patients with HCV genotype 2, SVR12 rates were 70% (7/10; 95% CI 35–93%) for 12 weeks and 92% (11/12; 95% CI 62–100%) for 24 weeks. Comparatively, the difference in SVR12 rate for the treatment groups overall was statistically significant (24 weeks compared with 12 weeks for all patients, $p = 0.023$); however, the differences in the SVR12 rates by genotype for the treatment groups were not statistically significant (for patients with genotype 1, $p = 0.0548$; for patients with genotype 2, $p = 0.4511$).

Results were similar between patients with and without cirrhosis in both treatment groups (Table 3). In the 12 week group, SVR12 rates were 82% (32/39) for those without cirrhosis and 83% (15/18) for those with compensated cirrhosis. In the 24-week group, they were 95% (37/39) in patients without cirrhosis and 100% (21/21) in those with cirrhosis.

The SVR12 rates for patients with genotype 1 HCV infection previously treated with both NS5A and NS3/4 inhibitors, including those who had also used NS5B inhibitor, were 86% (38/44) and 98% (40/41) in the 12- and 24-week groups, respectively. SVR12 rates were 86% (36/42) and 98% (39/40) with 12 and 24 weeks of treatment, respectively, in patients previously treated with daclatasvir plus asunaprevir, 100% (3/3) and 100% (11/11) in those previously treated with ledipasvir–sofosbuvir, and 100% (1/1) and 100% (4/4) in patients previously treated with daclatasvir plus asunaprevir and then ledipasvir–sofosbuvir. The SVR12 rates in the 12- and 24-week groups for patients with genotype 2 HCV infection previously treated with sofosbuvir were 67% (6/9) and 91% (10/11), respectively.

No patients had virologic nonresponse. A total of 11 patients relapsed, 9 of whom were in the 12-week grosup. One patient terminated treatment on day 8 because of an adverse event and did not achieve SVR12. In post hoc logistic regression analyses of relapse in the overall population (n = 116), the only factor that was statistically significant was treatment duration, where the likelihood for relapse was 5.5-fold higher with 12 weeks than with 24 weeks ($p = 0.0399$). For genotype 1 patients in both treatment groups (n = 95) and in the 12 week group alone (n = 47), no factor was statistically significant.

Viral resistance analyses

Among the 116 patients included in the resistance analysis population, the prevalence of baseline NS5A RASs was high and similar between the two treatment groups irrespective of genotype. Overall, 96% (54/56) in the 12-week group and 93% (56/60) in the 24-week group had baseline NS5A RASs. Most patients with genotype 1 HCV had 2 or more NS5A RASs (overall 85%, 80/94), including Y93

Table 1 Patient demographics and baseline characteristics

	Sofosbuvir-velpatasvir + ribavirin					
	Genotype 1		Genotype 2		Total	
	12 weeks (n = 47)	24 weeks (n = 48)	12 weeks (n = 10)	24 weeks (n = 12)	12 weeks (n = 57)	24 weeks (n = 60)
Mean (range) age, years	63 (38–81)	64 (35–79)	59 (21–76)	61 (46–70)	62 (21–81)	63 (35–79)
Female, n (%)	29 (62)	28 (58)	5 (50)	5 (42)	34 (60)	33 (55)
Race, n (%)						
Asian	47 (100)	48 (100)	10 (100)	12 (100)	57 (100)	60 (100)
Median (range) BMI, kg/m^2	24 (18–33)	23 (18–30)	23 (21–29)	24 (18–36)	24 (18–33)	23 (18–36)
Genotype, n (%)						
1	47 (100)	48 (100)	–	–	47 (82)	48 (80)
1a	2 (4)	1 (2)	–	–	2 (4)	1 (2)
1b	45 (96)	47 (98)	–	–	45 (79)	47 (78)
2	–	–	10 (100)	12 (100)	10 (18)	12 (20)
2a	–	–	7 (70)	8 (67)	7 (12)	8 (13)
2b	–	–	3 (30)	4 (33)	3 (5)	4 (7)
Mean (SD) HCV RNA, $\log_{10}$ IU/mL	6.2 (0.47)	6.2 (0.51)	6.6 (0.46)	6.2 (0.86)	6.3 (0.49)	6.2 (0.58)
HCV RNA $\geq$ 800,000 IU/mL, n (%)	37 (79)	38 (79)	9 (90)	8 (67)	46 (81)	46 (77)
No. of prior DAAs, n (%)						
1	2 (4)	0	9 (90)	8 (67)	11 (19)	8 (13)
2	34 (72)	39 (81)	1 (10)	2 (17)	35 (61)	41 (68)
$\geq$ 3	11 (23)	9 (19)	–	2 (17)	11 (19)	11 (18)
No. of prior treatment regimens, n (%)						
1	13 (28)	13 (27)	2 (20)	6 (50)	15 (26)	19 (32)
2	15 (32)	18 (38)	5 (50)	3 (25)	20 (35)	21 (35)
3	8 (17)	5 (10)	2 (20)	2 (17)	10 (18)	7 (12)
$\geq$ 4	11 (23)	12 (25)	1 (10)	1 (8)	12 (21)	13 (22)
Cirrhosis, n (%)						
Yes	16 (34)	18 (38)	2 (20)	3 (25)	18 (32)	21 (35)
No	31 (66)	30 (63)	8 (80)	9 (75)	39 (68)	39 (65)
Prior DAAs by class, n (%)						
NS5A + NS3 ± NS5B	44 (94)	41 (85)	1 (10)	2 (17)	45 (79)	43 (72)
NS5B ± NS3	–	–	9 (90)	9 (75)	9 (16)	9 (15)
NS5A ± NS5B	3 (6)	7 (15)	–	1 (8)	3 (5)	8 (13)
Prior DAAs, n (%)						
DCV	44 (94)	40 (83)	–	1 (8)	44 (77)	41 (68)
DCV + ASV	42 (89)	40 (83)	–	1 (8)	42 (74)	41 (68)
SOF	3 (6)	11 (23)	9 (90)	11 (92)	12 (21)	22 (37)
LDV-SOF	3 (6)	11 (23)	–	1 (8)	3 (5)	12 (20)
DCV + ASV and LDV–SOF	1 (2)	4 (8)	–	–	1 (2)	4 (7)
SMV					6	7
TVR					2	1
VAN					–	1
GRZ + ELB					1	1
OMB + PAR					1	1
GLE + PIB					1	–
IL-28B, n (%)						

Table 1 (continued)

	Sofosbuvir-velpatasvir + ribavirin					
	Genotype 1		Genotype 2		Total	
	12 weeks (n = 47)	24 weeks (n = 48)	12 weeks (n = 10)	24 weeks (n = 12)	12 weeks (n = 57)	24 weeks (n = 60)
CC	15 (32)	21 (44)	8 (80)	6 (50)	23 (40)	27 (45)
CT	28 (60)	20 (42)	1 (10)	6 (50)	29 (51)	26 (43)
TT	4 (9)	7 (15)	1 (10)	–	5 (9)	7 (12)
NS5A resistance-associated substitutions, *n/n* (%)						
Without	1/46 (2)	2/48 (4)	1/10 (10)	2/12 (17)	2/56 (4)	4/60 (7)
With	45/46 (98)	46/48 (96)	9/10 (90)	10/12 (83)	54/56 (96)	56/60 (93)
1	5/46 (11)	6/48 (13)	9/10 (90)	8/12 (67)	14/56 (25)	14/60 (23)
≥2	40/46 (87)	40/48 (83)	–	2/12 (17)	40/56 (71)	42/60 (70)
Y93 any ± other	41/46 (89)	39/48 (81)	–	–	41/56 (73)	39/60 (65)
L31 any ± other	38/46 (83)	42/48 (88)	9/10 (90)	10/12 (83)	47/56 (84)	52/60 (87)
P32 deletion ± other	2/46 (4)	3/48 (6)	–	–	2/56 (4)	3/60 (5)

ASV asunaprevir, *BMI* body mass index, *DAA* direct-acting antiviral, *DCV* daclatasvir, *ELB* elbasvir, *GLE* glecaprevir, *GT* genotype, GRZ grazoprevir, *HCV* hepatitis C virus, *LDV* ledipasvir, *OMB* ombitasvir, *PAR* paritaprevir, *PIB* pibrentasvir, *SMV* simeprevir, *SOF* sofosbuvir, *TVR* telaprevir, *VAN* vaniprevir

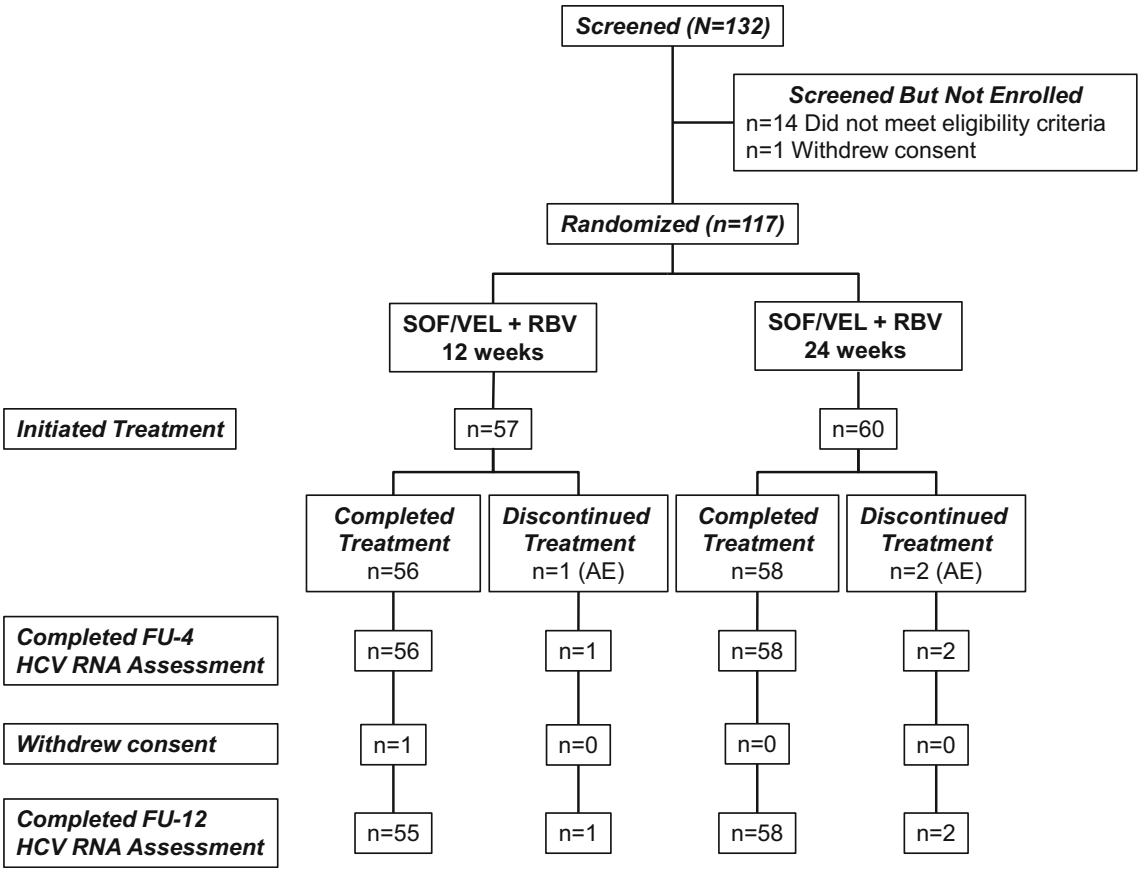

Fig. 1 Patient disposition throughout the study. *FU-4* follow-up week 4, *FU-12*, follow-up week 12, *HCV* hepatitis C virus, *RBV* ribavirin, *SOF* sofosbuvir, *VEL* velpatasvir

Table 2 Treatment response to sofosbuvir-velpatasvir + ribavirin

	Sofosbuvir-velpatasvir + ribavirin					
	Genotype 1		Genotype 2		Total	
	12 weeks (n = 47)	24 weeks (n = 48)	12 weeks (n = 10)	24 weeks (n = 12)	12 weeks (n = 57)	24 weeks (n = 60)
HCV RNA < 15 IU/mL, n/n (%)						
On treatment						
Week 1	12/47 (26)	11/48 (23)	0/10	4/12 (33)	12/57 (21)	15/60 (25)
Week 2	29/46 (63)	34/48 (71)	7/10 (70)	8/12 (67)	36/56 (64)	42/60 (70)
Week 4	45/46 (98)	47/48 (98)	10/10 (100)	12/12 (100)	55/56 (98)	59/60 (98)
Week 8	46/46 (100)	48/48 (100)	10/10 (100)	12/12 (100)	56/56 (100)	60/60 (100)
Week 12	46/46 (100)	47/47 (100)	10/10 (100)	12/12 (100)	56/56 (100)	59/59 (100)
Week 16	–	46/46 (100)	–	12/12 (100)	–	58/58 (100)
Week 24	–	46/46 (100)	–	12/12 (100)	–	58/58 (100)
After treatment						
Week 4	42/47 (89)	47/48 (98)	7/10 (70)	12/12 (100%)	49/57 (86)	59/60 (98%)
Week 12 (SVR12)	40/47 (85)	47/48 (98)	7/10 (70)	11/12 (92%)	47/57 (82)	58/60 (97%)
95% CI	72–94%	89–100%	35–93%	62–100%	70–91%	89–100%
Week 24 (SVR24)	40/47 (85)	47/48 (98)	7/10 (70)	11/12 (92%)	47/57 (82)	58/60 (97%)
95% CI	72–94%	89–100%	35–93%	62–100%	70–91%	89–100%
Virologic failure, n (%)						
On treatment	0	0	0	0	0	0
Relapse	6	1	3	1	9	2
Completed treatment	6	1	3	1	9	2
Discontinued treatment	0	0	0	0	0	0
Other virologic outcome, n (%)						
Did not complete treatment	1[a]	0	0	0	1	0

GT genotype, *HCV* hepatitis C virus, *SVR12* sustained virologic response 12 weeks after treatment

[a]Patient terminated participation on day 4 of treatment because of an adverse event (rash)

alone or in combination with other substitutions (overall 85%, 80/94) and P32 deletions (overall 5%, 5/94). The majority of those with a Y93 RAS also had L31 RAS (overall 89%, 71/80). Eighty-six percent (71/80) of patients with genotype 2 infection had 1 or 2 NS5A RASs at baseline (overall 86%, 19/22; genotype 2a 87%, 13/15; genotype 2b 86%, 6/7). All patients with genotype 2 infection and NS5A RAVs had L31M.

SVR12 was achieved in 85% (46/54) and 96% (54/56) of patients with baseline NS5A RASs in the 12- and 24-week groups, respectively (Table 3). Among those with two or more baseline NS5A RASs, 85% (34/40) in the 12-week group and 98% (41/42) in the 24-week group achieved SVR12. For patients with HCV genotype 1, SVR12 was achieved in 85% (35/41) and 100% (39/39) of those with any Y93 RAS, 82% (28/34) and 100% (37/37) for those with Y93 combined with L31 RASs, and 100% (2/2) and 67% (2/3) in patients with P32 deletions, in the 12- and 24-week groups, respectively. Among patients with genotype 2 infection with L31M RASs, 78% (7/9) and 90% (9/10) achieved SVR12 in the 12- and 24-week groups, respectively.

Seven patients (n = 4 HCV genotype 1b infection, n = 3 HCV genotype 2b infection) had NS5B RASs at baseline (n = 3 in the 12-week group and n = 4 in the 24-week group). All achieved SVR12.

None of the 11 patients who relapsed across the treatment groups developed treatment-emergent RASs at a cutoff of 15% or 1%.

Safety

Eighty-one percent (46/57) of patients in the 12-week group and 75% (45/60) of patients in the 24-week group experienced an adverse event (Table 4). The most commonly reported adverse events were viral upper respiratory tract infection (28%), anemia (23%), and headache (11%). Anemia was reported at similar percentages in the 12- and

Table 3 SVR12 by cirrhosis, prior direct-acting antivirals, and baseline resistance-associated substitutions

	Sofosbuvir-velpatasvir + ribavirin					
	Genotype 1		Genotype 2		Total	
	12 weeks	24 weeks	12 weeks	24 weeks	12 weeks weeks	24 weeks
Cirrhosis						
Yes	81% (13/16)	100% (18/18)	100% (2/2)	100% (3/3)	83% (15/18)	100% (21/21)
No	87% (27/31)	97% (29/30)	63% (5/8)	89% (8/9)	82% (32/39)	95% (37/39)
Prior DAAs by class						
NS5A + NS3 ± NS5B	86% (38/44)	98% (40/41)	100% (1/1)	100% (2/2)	87% (39/45)	98% (42/43)
NS5B ± NS3	–	–	67% (6/9)	89% (8/9)	67% (6/9)	89% (8/9)
NS5A ± NS5B	67% (2/3)	100% (7/7)	–	100% (1/1)	67% (2/3)	100% (8/8)
Prior DAAs						
DCV	84% (37/44)	98% (39/40)	–	100% (1/1)	84% (37/44)	98% (40/41)
DCV + ASV	86% (36/42)	98% (39/40)	–	100% (1/1)	86% (36/42)	98% (40/41)
SOF	100% (3/3)	100% (11/11)	67% (6/9)	91% (10/11)	75% (9/12)	96% (21/22)
LDV/SOF	100% (3/3)	100% (11/11)	–	100% (1/1)	100% (3/3)	100% (12/12)
DCV + ASV and LDV/SOF	100% (1/1)	100% (4/4)	–	–	100% (1/1)	100% (4/4)
NS3-containing regimens	50% (4/8)	100% (8/8)	–	0% (0/1)	50% (4/8)	89% (8/9)
Other DAA combinations	100% (2/2)	100% (1/1)	100% (1/1)	100% (1/1)	100% (3/3)	100% (2/2)
NS5A resistance-associated substitutions						
Without	100% (1/1)	100% (2/2)	0% (0/1)	100% (2/2)	50% (1/2)	100% (4/4)
With	87% (39/45)	98% (45/46)	78% (7/9)	90% (9/10)	85% (46/54)	96% (54/56)
1	100% (8/8)	100% (6/6)	78% (7/9)	88% (7/8)	86% (12/14)	93% (13/14)
≥ 2	85% (34/40)	98% (39/40)	–	100% (2/2)	85% (34/40)	98% (41/42)
Y93any ± other	85% (35/41)	100% (39/39)	–	–	85% (35/41)	100% (39/39)
L31any ± other	84% (32/38)	98% (41/42)	78% (7/9)	90% (9/10)	83% (39/47)	96% (50/52)
P32 deletion ± other	100% (2/2)	67% (2/3)	–	–	100% (2/2)	67% (2/3)

ASV asunaprevir, *DAA* direct-acting antiviral, *DCV* daclatasvir, *GT* genotype, *LDV* ledipasvir, *SOF* sofosbuvir

24-week treatment groups, 25% and 22%, respectively. Four patients, all in the 24-week group, experienced a Grade 3, serious adverse event; 2 had hepatocellular carcinoma, 1 had hepatic angiosarcoma, and 1 had pneumonia. None of the serious adverse events was considered related to study treatment.

Three patients had adverse events leading to premature discontinuation of treatment. One of them, in the 12-week group, discontinued on treatment day 8 because of rash and did not achieve SVR12. The rash was considered related to study treatment and resolved within 1 month. Another patient, in the 24-week group, had hepatic angiosarcoma that was considered unrelated to study treatment. This patient discontinued study drugs on day 97 of treatment and achieved SVR12. The third patient, also in the 24-week group, experienced moderately severe depression that was considered related to study treatment; the patient's medical history was notable for a prior episode of depression related to treatment with peginterferon plus ribavirin. This patient discontinued after 5 weeks of treatment and achieved SVR12.

Ten patients had adverse events that led to ribavirin dose reduction ($n = 9$) or interruption ($n = 1$). All ten patients had anemia that was considered related to study treatment, and one also had headache considered related to study treatment. Seven of the ten reached SVR12; three experienced relapse. All three had genotype 2 HCV and were in the 12-week group.

No patients had Grade 4 laboratory abnormalities. The only Grade 3 laboratory abnormalities that occurred in more than one patient were hyperglycemia ($n = 8$), lymphocyte reduction ($n = 8$), and decreased hemoglobin levels ($n = 6$). All eight patients with Grade 3 hyperglycemia had a history of diabetes.

Discussion

In this Phase 3 study in Japan, sofosbuvir–velpatasvir plus ribavirin was highly effective and well tolerated in patients with HCV genotype 1 or 2 infection with or without compensated cirrhosis who had not achieved sustained

Table 4 Adverse events and laboratory abnormalities

	Sofosbuvir–velpatasvir + ribavirin	
	12 weeks (n = 57)	24 weeks (n = 60)
No. (%) of patients with any adverse event	46 (81)	45 (75)
No. (%) of Grade 3 or 4 adverse events	0	4 (7)
No. (%) of patients with a serious adverse event	0	4 (7)
Adverse events leading to discontinuation of all study drug, n (%)	1 (2)	2 (3)
Deaths, n	0	0
Adverse events in $\geq$ 5% of patients in either treatment group, n (%)		
Upper respiratory tract viral infection	20 (35)	13 (22)
Anemia	14 (25)	13 (22)
Headache	11 (19)	2 (3)
Stomatitis	5 (9)	3 (5)
Eczema	4 (7)	2 (3)
Nausea	5 (9)	1 (2)
Pharyngitis	3 (5)	3 (5)
Pruritus	2 (4)	4 (7)
Back pain	4 (7)	1 (2)
Rash	2 (4)	3 (5)
Dry skin	0	4 (7)
Gastroenteritis	0	4 (7)
Malaise	1 (2)	3 (5)
Upper abdominal pain	3 (5)	0
Oral herpes	0	3 (5)
Upper respiratory tract inflammation	0	3 (5)
Serious adverse events, n (%)		
Hepatocellular carcinoma	0	2 (3)
Hepatic angiosarcoma	0	1 (2)
Pneumonia	0	1 (2)
Laboratory abnormalities (Grade 3 or above), n (%)		
Hyperglycemia, > 250 to 500 mg/dL	3 (5)	5 (8)
Lymphocytes, 350 to < 500/mm^3	1 (2)	7 (12)
Hemoglobin, 7.0 to < 9.0 g/dL or decrease $\geq$ 4.5 g/dL	2 (4)	4 (7)
Hyponatremia, 121 to < 125 mmol/L	0	1 (2)
Neutrophils, 500 to < 750/mm^3	0	1 (2)
Platelets, 25,000 to < 50,000/mm^3	0	1 (2)
White blood cells, 1000–1500/mm^3	0	1 (2)

virologic response after the previous treatment with DAA-containing regimens, including NS5A inhibitors. In this study, extending duration of therapy with sofosbuvir–velpatasvir plus ribavirin to 24 versus 12 weeks resulted in higher SVR rates, and the difference was statistically significant. In a univariate regression analysis of all enrolled patients, the only factor significantly associated with relapse was shorter treatment duration, suggesting that 24 weeks of treatment is of benefit for all DAA-experienced patients. The results with 24 weeks of treatment in the current study are similar to a smaller, prior study of 24 weeks of sofosbuvir–velpatasvir plus ribavirin in DAA-experienced patients, which resulted in SVR12 rates of 97% in patients with HCV genotype 1 and 93% in those with HCV genotype 2 [8]. However, only 7% of patients in the prior study were infected with HCV genotype 1b, compared with 78% in the current study, and only 14% had at least 1 NS5A RASs at baseline, compared with 92% of the HCV genotype 1 patients in the current study.

The adverse event profile in this study was generally similar to those reported in the previous studies of regimens including sofosbuvir and ribavirin [9–12]. Three

patients (2.6%) discontinued treatment because of an adverse event, yet despite the early discontinuation, 2 of them achieved SVR12. Typical with ribavirin-containing regimens, anemia occurred in approximately one-fifth of patients but did not result in treatment discontinuation in any patients.

The current Japanese treatment guidelines recommend glecaprevir-pibrentasvir as the first-line retreatment option for patients who have failed NS3/4A protease inhibitor and NS5A inhibitor, and who do not have baseline NS3/4 or NS5A RASs. The Phase 3 CERTAIN-1 study evaluated treatment with glecaprevir-pibrentasvir for 12 weeks in Japanese patients [13]. Of the 33 DAA-experienced subjects, 30 had previously been treated with daclatasvir and asunaprevir, 2 with peginterferon and ribavirin and simeprevir, and 1 with sofosbuvir and ribavirin. SVR12 was achieved by 94% (31/33) of patients, and both patients with virologic failure had genotype 1b HCV infection and P32 deletions in the NS5A region at baseline. One of the two patients with virologic failure also had the NS3 RAS D168V at baseline and emergent A156D/V at failure. In the United States, glecaprevir-pibrentasvir is not recommended for HCV genotype 1 patients who previously received both NS5A and NS3/4A inhibitors, and instead sofosbuvir–velapatasvir–voxilaprevir is recommended [14]. One clear benefit of sofosbuvir–velpatasvir plus ribavirin is that it can be used in patients with decompensated cirrhosis.

The previous studies have shown that patients with genotype 1b infection who were unsuccessfully treated with daclatasvir plus asunaprevir frequently have complex RAS profiles [3, 4]. Similar observations were made in this study, as the majority of genotype 1 patients had 2 or more NS5A RASs at baseline. Specific NS5A RASs associated with daclatasvir plus asunaprevir treatment failures that confer high levels of resistance to NS5A inhibitors include dual mutations at Y93 and L31 as well as P32 deletions. The dual NS5A RASs and P32 deletions have been associated with relapse in ledipasvir–sofosbuvir and glecaprevir-pibrentasvir re-treatment studies [13, 15–17]. In this study, the overall presence of NS5A substitutions or the presence of specific NS5A substitutions at baseline had no discernible effect on the rates of SVR12 with sofosbuvir–velpatasvir plus ribavirin. All 37 patients in the 24-week group with baseline Y93 and L31 RASs achieved SVR12. Furthermore, 4 of the 5 patients enrolled in the current study with a P32 deletion at baseline achieved SVR with 12 or 24 weeks of treatment.

The majority of patients in the current study with genotype 2a (87% [13/15]) or genotype 2b (86% [6/7]) had 1 or more NS5A RASs at baseline, all with L31M. In contrast, it was previously reported that worldwide 97% of patients with HCV genotype 2a and 39% of patients with HCV genotype 2b had L31M [18]. Our data suggest that there may be a higher prevalence of L31M in HCV genotype 2b strains circulating in Japan relative to the global population, although this is based on a small number of patients.

Prior studies have suggested that there is an association between the duration of prior DAA treatment and success in retreatment, with patients treated with shorter durations of all-oral NS5A inhibitor-based DAA therapy (4–8 weeks) having higher retreatment SVR rates compared to those initially treated for longer durations (10–12 weeks) [19, 20], a phenomenon, perhaps, resulting from greater virologic resistance developing during longer treatment. In the current study, the median duration of most recent prior DAA treatment was 12–14 weeks, and 95% of patients had baseline NS5A RASs. The high SVR12 (97%) rate in among patients who received 24 weeks of treatment demonstrates that the inclusion of ribavirin and the extended treatment duration are effective in treating this highly treatment-experienced patient population infected with resistant HCV.

This study was designed to evaluate two durations of treatment with the same regimen of sofosbuvir–velpatasvir and ribavirin. It did not include a ribavirin-free arm, because the population consisted of DAA-experienced patients expected to have complex resistance profiles who would benefit from ribavirin in addition to two highly potent direct-acting antivirals. As such, the study does not give insight into whether the addition of ribavirin could be unnecessary for some patients. The sample size precludes meaningful analyses of subgroups of patients.

Further limitations of this study are the small number of patients with genotype 1a or genotype 2 HCV infections. The distribution of genotypes and subtypes is representative of the HCV population in Japan, which is predominantly genotype 1b [21]. The small sample size of HCV genotype 2 patients makes it difficult to interpret the high rate of relapse with 12 weeks of treatment, which does not seem to be attributable to the presence of L31M RASs nor the presence of cirrhosis. The three patients with genotype 1a infection were all successfully treated in the current study; however, the sample size is too small to predict treatment outcomes in a larger population with this subtype. Another limitation of the study is that there were few patients who had previously been treated with other next-generation DAA regimens, such as glecaprevir–pibrentasvir ($n = 1$), elbasvir–grazoprevir ($n = 2$), and ritonavir-boosted ombitasvir–pariteprevir ($n = 2$); all of these patients were successfully treated (data not shown).

In summary, sofosbuvir–velpatasvir plus ribavirin for 24 weeks was highly effective and well tolerated in Japanese patients with chronic HCV genotype 1 or 2 infection who previously failed treatment with a DAA. The presence of NS5A or NS5B RASs at baseline, including those

associated with virologic failure with other DAA regimens, did not impact treatment outcomes. Sofosbuvir–velpatasvir plus ribavirin for 24 weeks is an effective salvage regimen for this population with limited treatment options.

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Compliance with ethical standards

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Efficacy and safety of sofosbuvir–velpatasvir with or without ribavirin in HCV-infected Japanese patients with decompensated cirrhosis: an open-label phase 3 trial

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Abstract

Background In Japan, hepatitis C virus (HCV)-infected patients with decompensated cirrhosis currently have no treatment options. In this Phase 3 study, we evaluated sofosbuvir–velpatasvir with or without ribavirin for 12 weeks in patients with any HCV genotype and decompensated cirrhosis [Child–Pugh–Turcotte (CPT) class B or C] in Japan.

Methods Patients were randomized 1:1 to receive sofosbuvir–velpatasvir with or without ribavirin for 12 weeks. Randomization was stratified by CPT class and genotype. Sustained virologic response 12 weeks following completion of treatment (SVR12) was the primary efficacy endpoint.

Results Of the 102 patients enrolled, 57% were treatment naive, 78% and 20% had genotype 1 and 2 HCV infection, respectively, and 77% and 20% had CPT class B and C cirrhosis, respectively, at baseline. Overall, 61% of patients were female and the mean age was 66 years (range 41–83). SVR12 rates were 92% (47/51) in each group. Among patients who achieved SVR12, 26% had improved CPT class from baseline to posttreatment week 12. Most adverse events (AEs) were consistent with clinical sequelae of advanced liver disease or known toxicities of ribavirin. Four patients (8%) who received sofosbuvir–velpatasvir and seven (14%) who received sofosbuvir–velpatasvir plus ribavirin experienced a serious AE. The 3 deaths (bacterial sepsis, gastric varices hemorrhage, hepatocellular carcinoma) were attributed to liver disease progression.

Conclusion Sofosbuvir–velpatasvir for 12 weeks provides a highly effective and well-tolerated therapy for Japanese

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patients with HCV and decompensated cirrhosis. Ribavirin did not improve efficacy but increased toxicity.

Keywords Sofosbuvir · Velpatasvir · Decompensated cirrhosis · Advanced liver disease · Direct-acting antivirals

Abbreviations

AE	Adverse event
BMI	Body mass index
CI	Confidence interval
CPT	Child–Pugh–Turcotte
DAA	Direct-acting antiviral
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
LLOQ	Lower limit of quantification
MELD	Model for end-stage liver disease
NI	Nucleoside inhibitor
RAS	Resistance-associated substitution
RNA	Ribonucleic acid
SAE	Serious adverse event
SVRxx	Sustained virologic response at "xx" weeks following completion of treatment

Introduction

Globally, the treatment of HCV infection has been transformed with the development of direct-acting antiviral (DAA) agents, which target viral proteins and cellular processes essential to viral replication. These interferon-free, DAA-based regimens are generally well-tolerated and result in high rates of sustained virologic response (SVR) across most patient populations. However, some regimens containing protease inhibitors have been associated with hepatotoxicity and hepatic decompensation, particularly in patients with advanced cirrhosis thus precluding their use in some patients, including those with decompensated cirrhosis [1]. In contrast, ledipasvir/sofosbuvir and sofosbuvir/velpatasvir have demonstrated both safety and efficacy in patients with decompensated liver disease [2–4]. These studies were conducted in North America, Europe, Australia, and New Zealand. Data are lacking in Japanese patients, and there are no approved antiviral therapies currently available for this population in Japan. The current Japan Society of Hepatology (JSH) guidelines therefore do not recommend the use of DAA agents in patients with decompensated cirrhosis due to lack of safety or efficacy data in Japanese patients [5].

Of the approximately 1.0–1.5 million people chronically infected with hepatitis C virus (HCV) in Japan [5], approximately 35,000–50,000 may have decompensated cirrhosis [6, 7]. Liver transplantation is a potential nonpharmaceutical intervention; however, it is not commonly done in Japan, with only 438 liver transplants performed in 2016 [8]. Patients with decompensated cirrhosis are at high risk for development of hepatocellular carcinoma (HCC), bleeding diatheses, and fulminant infections. One-year survival rates in patients with Child–Pugh–Turcotte (CPT) class B or CPT class C cirrhosis are 80% and 45%, respectively [9, 10]. A retrospective cohort study of Japanese patients with CPT class C cirrhosis on the liver transplant registry demonstrated a mean survival time of less than 16 months and 2-year survival probability was less than 40% [11]. Without available antiviral therapy and limited options for liver transplantation in Japan [8], the prognosis for Japanese patients with chronic HCV infection and decompensated cirrhosis is poor. A safe and effective HCV treatment will address the unmet medical need for this population.

Sofosbuvir–velpatasvir (400/100 mg) is a fixed-dose combination that combines 2 DAAs. Sofosbuvir is a nucleotide analog that is a potent, pangenotypic and selective NS5B polymerase inhibitor, and velpatasvir is a potent, pangenotypic, next-generation HCV NS5A inhibitor. Sofosbuvir–velpatasvir is approved in the US, European Union, and other regions for the treatment of genotypes 1–6 chronic HCV infection in patients with and without compensated cirrhosis and for use with ribavirin in patients with decompensated cirrhosis [12, 13].

The ASTRAL-4 study evaluated 12 and 24 weeks of treatment with sofosbuvir–velpatasvir with or without ribavirin in HCV-infected patients with CPT class B decompensated cirrhosis in the US [4]. Rates of sustained virologic response 12 weeks post treatment (SVR12) were 83% in patients who received 12 weeks of sofosbuvir–velpatasvir, 94% in patients who received 12 weeks of sofosbuvir–velpatasvir plus ribavirin, and 86% in patients who received 24 weeks of sofosbuvir–velpatasvir. Notably, the numeric difference in SVR12 rates in genotype 1b and genotype 2 HCV-infected patients who received sofosbuvir–velpatasvir for 12 weeks or sofosbuvir–velpatasvir with ribavirin for 12 weeks did not differ substantially.

In this Phase 3 study, we evaluated the efficacy and safety of the fixed-dose combination tablet of sofosbuvir–velpatasvir with or without ribavirin for 12 weeks in Japanese HCV-infected patients with decompensated cirrhosis.

Methods

Patients

Eligible patients were 20 years of age and older with chronic HCV infection, quantifiable HCV RNA at screening, and CPT score 7–12, inclusive. The calculation of the

CPT score at screening used either the international normalized ratio or prothrombin activation percentage for the coagulation parameter, at the investigator's discretion (Supplemental Table 1). Patients were to have liver imaging within 4 months of baseline to exclude HCC. Patients were excluded from this study if they had a positive test result for hepatitis B surface antigen or human immunodeficiency virus, had HCC within 2 years prior to screening, any recurrence of HCC after curative treatment (e.g., successful treatment with surgical resection or radiofrequency ablation), prior treatment with an NS5A inhibitor, or creatinine clearance < 50 mL/min as calculated by the Cockcroft–Gault equation using actual body weight. Use of concomitant amiodarone was prohibited from 60 days prior to day 1 and throughout the treatment period. Full eligibility criteria are provided in the supplementary information.

Study design and randomization

This was a Phase 3, multicenter, open-label study. Via an interactive web response system, patients were randomly assigned 1:1 to sofosbuvir–velpatasvir with or without ribavirin for 12 weeks. Randomization was stratified by genotype (genotype 1 vs. non-genotype 1) and CPT class at screening (CPT class B vs C). For the purposes of randomization, a patient with nondefinitive or mixed HCV genotype results was considered non-genotype 1. Across the study population, at least 15 patients were to have non-genotype 1 HCV infection and approximately 10% of patients were to have CPT class C cirrhosis. Enrollment of patients with CPT class C cirrhosis began after an independent data monitoring committee evaluated the safety data through 4 weeks of treatment from the first 20 patients with CPT class B cirrhosis.

Sofosbuvir–velpatasvir (400/100 mg) fixed-dose combination was administered once daily. Ribavirin (REBETOL, MSD KK) was administered with food twice daily. For patients with CPT class B cirrhosis at screening dosing was based on body weight (600 mg daily in patients ≤ 60 kg, 800 mg for patients > 60–80 kg, and 1000 mg for those > 80 kg). All patients with CPT class C cirrhosis received 600 mg daily regardless of weight.

All patients provided written informed consent to participate, and the study was conducted consistent with the ethical standards, including but not limited to the International Council for Harmonisation guideline for Good Clinical Practice, the original principles embodied in the Declaration of Helsinki, and the J-GCP (Ministerial Ordinance on Good Clinical Practice for Drugs). This study was approved by an institutional review board at each study site prior to the initiation of any screening or study-specific procedures.

Study assessments

Screening assessments included HCV genotyping, *IL28B* genotyping, and standard laboratory and clinical tests. HCV genotype and subtype were determined using the Siemens VERSANT HCV Genotype INNO-LiPA2.0 Assay. *IL28B* genotype was determined by polymerase chain reaction amplification of the single-nucleotide polymorphism rs12979860, with sequence-specific forward and reverse primers and allele-specific fluorescently labeled TaqMan minor groove binder probes. Plasma HCV RNA levels were evaluated at screening; at day 1 of treatment, at weeks 2, 4, 8, and 12 during treatment, and at weeks 4, 12, and 24 after the end of treatment. HCV RNA levels were quantified using the COBAS Ampliprep/COBAS TaqMan HCV Test, v2.0 (Roche Molecular Systems, Inc., Branchburg, NJ), which has a lower limit of quantification (LLOQ) of 15 IU/mL.

Deep sequencing of the HCV NS5A and NS5B genes was performed for all patients at baseline and from those with virologic failure at the time of failure (DDL Diagnostic Laboratory, Rijswijk, Netherlands). RASs present in more than 15% of the sequence reads are reported. The resistance analysis population is comprised of patients with viral sequence data and virologic outcome data available.

Safety assessments included monitoring of adverse events (AEs) and clinical laboratory tests at all on-treatment visits; AEs were also collected up to 30 days after the last dose of study drug. Samples for clinical laboratory tests were collected at each posttreatment visit (4, 12, and 24 weeks after the last dose of study drug). All AEs and laboratory values were graded according to a standardized scale and AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 20.1.

Endpoints

The primary efficacy endpoint was SVR12, defined as HCV RNA $<$ LLOQ (i.e., < 15 IU/mL) 12 weeks after the end of treatment. Secondary efficacy endpoints included the change from baseline in the CPT and MELD scores at 12 weeks after end of treatment. CPT score for all baseline and post-baseline visits were calculated using prothrombin activation percentage for the coagulation parameter. The primary safety endpoint was discontinuation of study drugs due to AEs.

Statistical analysis

Point estimates with 2-sided 95% exact confidence intervals (CIs) for SVR12 based on the Clopper–Pearson method were provided for each treatment group. In the primary efficacy analysis, the SVR12 rate for patients in

Table 1 Baseline demographics and disease characteristics

	Sofosbuvir–velpatasvir 12 weeks $N = 51$	Sofosbuvir–velpatasvir plus ribavirin 12 weeks $N = 51$
Mean age (range) (years)	66 (43, 82)	66 (41, 83)
Female sex	33 (65)	29 (57)
Mean body mass index (range) (kg/m^2)	26.5 (20.4, 43.0)	25.8 (18.3, 58.6)
HCV genotype and subtype		
Genotype 1	41 (80)	39 (76)
Genotype 1a	1 (2)	0
Genotype 1b	40 (78)	39 (76)
Genotype 2	9 (18)	11 (22)
Genotype 2 (no confirmed subtype)	5 (10)	5 (10)
Genotype 2a	0	2 (4)[a]
Genotype 2a/2c	2 (4)	1 (2)
Genotype 2b	2 (4)	4 (8)
Genotype 3b	1 (2)	0
Mean HCV RNA (range) ($\log_{10}$ IU/mL)	5.7 (3.7–7.1)	5.8 (4.2–7.0)
IL28B CC genotype	33 (65)	37 (73)
CPT B [7–9][b]	40 (78)	39 (76)
MELD score $\leq$ 15	46 (90)	48 (94)
Ascites		
None	19 (37)	16 (31)
Mild/moderate	32 (63)	33 (65)
Severe	0	2 (4)
Encephalopathy		
None	23 (45)	22 (43)
Medication-controlled	28 (55)	29 (57)
No prior HCV treatment	27 (53)	31 (61)
Mean estimated glomerular filtration rate (range) (mL/min)[c]	93 (40, 183)	89 (42, 299)

Data presented are *n* (%) unless stated otherwise

CPT Child–Pugh–Turcotte

[a]One patient with missing HCV genotype was subsequently determined to have genotype 2a HCV infection by BLAST analysis

[b]The CPT score was calculated using prothrombin activation percentage for the coagulation parameter

[c]The estimated glomerular filtration rate was calculated using the Cockcroft–Gault equation

each treatment group was compared to the spontaneous clearance rate of 1% using a 2-sided exact 1-sample binomial test with Bonferroni alpha adjustment (each at the 0.025 significance level).

Results

Baseline characteristics and disposition

Demographics and baseline characteristics are presented in Table 1. Of 155 patients screened, a total of 102 patients were enrolled at 33 sites in Japan, of which 100 (98%) completed treatment (Supplemental Fig. 1). All 53 patients who were excluded from study participation did not meet eligibility criteria (Supplemental Table 2). Demographics and baseline characteristics of the patients enrolled were generally balanced across both treatment groups and consistent with an older population with advanced liver disease. Overall, most patients were female (61%). The mean age was 66 years (range 41–83), and 58% were $\geq$ 65 years of age. Most patients had *IL28B* CC genotype (69%) and were treatment naive (57%). Among the 44 treatment-experienced patients, only 1 had previously been treated with a DAA (simeprevir in combination with peginterferon alfa-2a and ribavirin for 23 weeks); all others had been treated with interferon alone or in combination with ribavirin.

Table 2 Virologic response during and after treatment

	Sofosbuvir–velpatasvir 12 weeks $N = 51$	Sofosbuvir–velpatasvir plus ribavirin 12 weeks $N = 51$
HCV RNA < 15 IU/mL, *n*/*n* (%)		
On treatment		
Week 2	23/51 (45)	26/51 (51)
Week 4	49/51 (96)	46/51 (90)
Week 8	51/51 (100)	49/51 (96)
Week 12	51/51 (100)	49/49 (100)
After treatment		
Week 4 (SVR4)	48/51 (94)	49/51 (96)
Week 12 (SVR12)	47/51 (92)	47/51 (92)
95% CI	81–98	81–98
Relapse after the end of treatment	4 (8)	2 (4)
Discontinued treatment due to adverse events	0	2 (4)

Overall, 80 patients (78%) had genotype 1 HCV infection [1 patient (1%) had HCV genotype 1a and 79 (77%) patients had HCV genotype 1b], 20 patients (20%) had genotype 2 HCV infection, and 1 patient (1%) had genotype 3 HCV infection. There was 1 patient who had an HCV genotype that was unable to be determined by LiPA or NS5B Sanger, but was later determined to have genotype 2a HCV infection by BLAST analysis. At baseline, 77% of patients were CPT class B (score 7–9), 20% were CPT class C (score 10–12), and 3% were CPT class A (score 6).

Efficacy

Virologic response

The SVR12 rates were 92% (47/51; 95% CI 81–98%) in each treatment group (Table 2). Both treatment groups met their primary efficacy endpoints with SVR12 rates that were statistically superior compared with the spontaneous clearance rate of 1% ($p < 0.001$).

When examined by genotype, SVR12 rates were high for patients with genotype 1 or 2 regardless if they received 12 weeks of sofosbuvir–velpatasvir or sofosbuvir–velpatasvir plus ribavirin (rates ranged from 89 to 100%, Table 3). The 1 patient with genotype 3 HCV infection in the study who was randomized to the sofosbuvir–velpatasvir group did not achieve SVR12. When examined by baseline CPT class, SVR12 rates were high in patients with CPT class B cirrhosis (≥ 95%) in both treatment groups (Table 3). Of the patients with baseline CPT class C cirrhosis, 80% (8/10) and 70% (7/10) in the sofosbuvir–velpatasvir and sofosbuvir–velpatasvir plus ribavirin groups, respectively, achieved SVR12.

Table 3 Rates of SVR12 by subgroup

	Sofosbuvir–velpatasvir 12 weeks $N = 51$	Sofosbuvir–velpatasvir plus ribavirin 12 weeks $N = 51$
Overall SVR12	47/51 (92)	47/51 (92)
Genotype		
1a	0/1 (0)	–
1b	39/40 (98)	35/39 (90)
2	8/9 (89)	12/12 (100)[a]
3	0/1 (0)	–
Baseline CPT class		
A	1/1 (100)	2/2 (100)
B	38/40 (95)	38/39 (97)
C	8/10 (80)	7/10 (70)

[a]Includes 1 patient who was initially categorized as missing HCV genotype, and subsequently determined to have genotype 2a by BLAST analysis

A total of 8 patients did not achieve SVR12, with 6 patients experiencing virologic relapse (Supplemental Table 3). No patients had virologic non-response. In the sofosbuvir–velpatasvir group, 4 of 51 patients (8%) relapsed. In the sofosbuvir–velpatasvir plus ribavirin group, 4 of 51 patients (8%) did not achieve SVR12. Of these 4 patients, 2 relapsed and 2 discontinued treatment early due to AEs and subsequently died.

Table 4 Shift of CPT class from baseline to posttreatment week 12

Posttreatment week 12 CPT class, *n* (%)	Overall *N* = 94		
	Baseline CPT class		
	CPT A (5–6) *N* = 3	CPT B (7–9) *N* = 76	CPT C (10–15) *N* = 15
CPT A (5–6)	3 (100)	19 (25)	0
CPT B (7–9)	0	55 (72)	5 (33)
CPT C (10–15)	0	2 (3)	10 (67)

CPT Child–Pugh Turcotte

Table 5 Adverse events and grade 3 and 4 laboratory abnormalities

	Sofosbuvir–velpatasvir 12 weeks *N* = 51	Sofosbuvir–velpatasvir plus ribavirin 12 weeks *N* = 51
Number (%) of patients experiencing any		
Adverse event	35 (69)	44 (86)
Grade 3 or above adverse event	2 (4)	5 (10)
Serious adverse event	4 (8)	7 (14)
Adverse event leading to discontinuation of sofosbuvir/velpatasvir	0	2 (4)
Adverse event leading to discontinuation of ribavirin	N/A	9 (18)
Adverse event leading to modification or interruption of ribavirin	N/A	18 (35)
Deaths	0	3 (6)
Common adverse events (≥ 10% either group)		
Anemia	0	20 (39)
Nasopharyngitis	7 (14)	3 (6)
Diarrhea	0	7 (14)
Laboratory abnormalities (≥ 10% either group)		
Hemoglobin < 10 g/dL	2 (4)	7 (14)
Lymphocytes, < 500/mm^3	0	5 (10)
Platelets, 25,000–50,000/mm^3	1 (2)	6 (12)
Hyperglycemia, > 250–500 mg/dL	5 (10)	9 (18)
Total bilirubin, > 2.5 × ULN	6 (12)	12 (24)

Toxicity grade must have increased at least 1 toxicity grade from baseline value (missing was considered grade 0) to be included. Patients were counted once at maximum toxicity grade for each laboratory test. Data were included up to the last dose date of any study drug + 30 days

Changes in liver function

Of all patients who achieved SVR12 in either arm, 26% (24/91) improved in CPT class and 2% (2/91) worsened in CPT class from baseline to posttreatment week 12 (Table 4). Improvement in CPT score was primarily driven by increase in albumin levels with 79% of the patients with improved CPT scores having increase in albumin (Supplemental Table 4). Similar changes were observed in MELD score with 27% (25/94) having improved MELD score and 15% (14/94) with worsening MELD score.

Analysis of resistance

Among the 100 patients included in the resistance analysis population, 41% (41/100) had baseline NS5A RASs. No patient had NS5B nucleoside inhibitor (NI) RASs.

In the sofosbuvir–velpatasvir group, 97% (33/34) of patients without baseline NS5A RASs and 82% (14/17) of patients with baseline NS5A RASs achieved SVR12. Of the 41 patients with genotype 1 HCV infection, there was 1 patient without baseline NS5A RASs and 1 patient with baseline NS5A RASs who relapsed. In the sofosbuvir–velpatasvir plus ribavirin group, 96% (24/25) of patients

without baseline NS5A RASs and 96% (23/24) of patients with baseline NS5A RASs achieved SVR12. Of the 37 patients with genotype 1 HCV infection, there was 1 patient without baseline NS5A RASs and 1 patient with baseline NS5A RASs who relapsed.

Of the 6 patients who experienced virologic relapse across both treatment groups, 4 had treatment-emergent NS5A RASs. No patient in either treatment group had NS5B NI RASs detected at baseline or relapse.

Safety

More patients treated with sofosbuvir–velpatasvir plus ribavirin experienced AEs (86%, 44/51) compared with patients treated with sofosbuvir–velpatasvir (69%, 35/51) (Table 5). No consistent, clinically significant trends were observed when looking at AE rates by CPT class, nor by age group.

Despite all the patients in the study having advanced liver disease, most AEs reported in this study were Grade 1 (mild) or Grade 2 (moderate) in severity. The most common AEs in the sofosbuvir–velpatasvir group were nasopharyngitis (14%) and in the sofosbuvir–velpatasvir plus ribavirin group they were anemia (39%) and diarrhea (14%).

Patients in the sofosbuvir–velpatasvir plus ribavirin group experienced AEs consistent with ribavirin toxicity. Eighteen of 51 patients (35%) had AEs that led to modification or interruption of ribavirin and 9 patients (18%) had AEs that led to discontinuation of ribavirin, with anemia being the most common in both instances.

Four patients (8%) in the sofosbuvir–velpatasvir group and 7 patients (14%) in the sofosbuvir–velpatasvir plus ribavirin group had serious adverse events (SAEs), and most were not considered treatment-related by the investigator (Supplemental Table 5). The only SAEs that occurred in > 1 patient were femur fracture (2 in the sofosbuvir–velpatasvir plus ribavirin group) and hepatic encephalopathy (1 in the sofosbuvir–velpatasvir group, 2 in the sofosbuvir–velpatasvir plus ribavirin group). Two of the three SAEs of hepatic encephalopathy occurred in patients with CPT class C cirrhosis.

Three patients in the study developed HCC, all of whom were diagnosed following treatment (on posttreatment day 1, posttreatment day 70 and posttreatment day 124). Two of the patients had CPT class B at baseline and one had CPT class C. The investigator did not consider these events related to study drug. There were 4 patients enrolled who had a history of HCC, none of whom experienced recurrence during the study.

Three deaths occurred during the study and all 3 patients received treatment with sofosbuvir–velpatasvir plus ribavirin. The ages of the patients who died were 51, 59 and 67 years; all 3 patients had CPT class C at baseline. Two of these patients discontinued study drugs early due to AEs not related to treatment. All 3 deaths occurred after treatment was stopped (posttreatment days 5 and 17 for the 2 patients that discontinued study drugs prematurely, and posttreatment day 158 for the patient that completed 12 weeks of study treatment). All of the deaths were due to progression of end-stage liver disease (septicemia, portal hypertension leading to gastrointestinal bleeding, and HCC) and none were considered to be related to study drugs by the investigator (Supplemental Table 6). No other patients discontinued sofosbuvir–velpatasvir in the study.

Fewer patients in the sofosbuvir–velpatasvir group had Grade 3 or 4 laboratory abnormalities compared with the sofosbuvir–velpatasvir plus ribavirin group (27 vs 53%, respectively) (Table 5). The observed laboratory abnormalities were consistent with those expected in a population with decompensated liver disease and, in the sofosbuvir–velpatasvir plus ribavirin group, consistent with the known toxicities of ribavirin. Post-baseline hemoglobin values < 10 g/dL were observed in 2 patients (4%) in the sofosbuvir–velpatasvir group and 7 patients (14%) in the sofosbuvir–velpatasvir plus ribavirin group. Additional information about laboratory abnormalities is provided in the supplementary information (Supplemental Fig. 2).

Discussion

In this Phase 3 study conducted in Japan, sofosbuvir–velpatasvir for 12 weeks was highly effective and generally safe and well-tolerated in patients with decompensated cirrhosis. The current study enrolled mostly patients with genotype 1b or 2, consistent with the Japanese population of HCV-infected patients. The identical SVR12 rates of 92% in the 2 treatment groups suggest that addition of ribavirin to sofosbuvir–velpatasvir did not improve efficacy for Japanese patients with decompensated cirrhosis. These results were comparable to those for the similar subpopulation enrolled in the ASTRAL-4 study, in which 12 weeks of treatment with sofosbuvir–velpatasvir without ribavirin resulted in SVR12 rates of 89% (16 of 18) and 100% (4 of 4) in patients with genotype 1b and 2, respectively [4]. Of note, the addition of ribavirin was most beneficial in patients with genotype 3 HCV infection in the ASTRAL-4 study, where the response was 35% higher in the group who received ribavirin (85%, 11 of 13 patients) compared to those who did not in either the sofosbuvir–velpatasvir 12 week group (50%, 7 of 14 patients) or 24 week group (50%, 6 of 12 patients).

Clinical attention to safety is appropriate in this patient population with advanced liver disease with high expected morbidity and mortality. In the current study, the AE

profile was consistent with the clinical sequelae of advanced liver disease and with the known toxicities of ribavirin. In the sofosbuvir–velpatasvir plus ribavirin group, 49% of patients needed significant modifications to their ribavirin dosing, primarily due to anemia. Overall sofosbuvir–velpatasvir was well-tolerated with the majority of AEs being Grade 1 or 2. Only 2 patients, both in the sofosbuvir–velpatasvir plus ribavirin group, discontinued sofosbuvir–velpatasvir for AEs that were not considered related to study drugs; both of these patients subsequently died due to progression of their liver disease. The safety profile observed in the current study, including the rate of deaths, was consistent with those observed in previous overseas trials of sofosbuvir–velpatasvir with and without ribavirin as well as ledipasvir–sofosbuvir with ribavirin in larger populations of patients with decompensated cirrhosis, despite the fact that the mean age of patients in the current study was 8–9 years older than in the overseas studies [2–4].

As interferon-free DAA-based regimens have only recently become available for the treatment of HCV, the clinical benefits of their use in patients with decompensated cirrhosis are being characterized. Achievement of SVR12 is associated with early improvements in liver function, as demonstrated by reductions in CPT and MELD scores through posttreatment week 12, in both the current study as well as previous studies of sofosbuvir-based regimens in this population [2–4]. In terms of long-term benefits of achieving SVR with DAA-based regimens in patients with decompensated cirrhosis, several studies have compared the survival rates of patients successfully treated with sofosbuvir-based regimens to historical matched controls from transplant waitlists and have demonstrated a decrease in mortality [14, 15]. There is also a growing body of literature demonstrating a reduction in risk of de novo HCC, consistent with observations in the interferon era [16–18].

Our study has several limitations, mostly related to characteristics of the enrolled patients. Although representative of the Japanese HCV-infected patient population, there was a lack of genotype diversity. The study included few patients with more severe cirrhosis (CPT class C) and none with baseline CPT score greater than 12. Patients who had been previously treated with DAAs were not included. Lastly, although early improvements in liver function were demonstrated through the study posttreatment period, the long-term clinical benefit of achievement of SVR in patients with decompensated liver disease can only be demonstrated through follow-up of the patients after the study.

In conclusion, treatment with sofosbuvir–velpatasvir for 12 weeks is the optimal regimen for Japanese patients with decompensated cirrhosis. The SVR12 rate was high regardless of genotype or CPT class. Addition of ribavirin to the regimen did not improve efficacy and was associated with more adverse events and laboratory abnormalities.

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Compliance with ethical standards

Conflict of interest Tetsuo Takehara has received honoraria and commercial research funding from Gilead. Naoya Sakamoto has received honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme, and Gilead, and has received commercial research funding from Gilead, AbbVie, Bristol-Myers Squibb, Merck Sharp & Dohme, Otsuka, and Shionogi. Shuhei Nishiguchi has received honoraria from Gilead, and has received commercial research funding from Gilead, Toray and Merck Sharp & Dohme. Yoshiyuki Ueno received commercial research funding from Gilead, Bristol-Myers Squibb, AbbVie, and Merck Sharp & Dohme. Hiroshi Yatshuhashi has received commercial research funding from Chugai. Tatsuo Kanda has received commercial research funding from AbbVie, Merck Sharp & Dohme, Chugai, and Sysmex. Minoru Sakamoto has received honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme, and Gilead, and has received commercial research funding from Gilead, AbbVie, Bristol-Myers Squibb, Merck Sharp & Dohme, Otsuka, and Shionogi. Akihiro Tamori has received honoraria from Gilead. Kazuaki Chayama has received honoraria from AbbVie, Merck Sharp & Dohme, Bristol-Myers Squibb, and Gilead. Gulan Zhang, Shampa De-Oertel, Hadas Dvory-Sobol, Takuma Matsuda, Luisa M. Stamm, and Diana M. Brainard are employees of and hold stock in Gilead Sciences. Yasuhito Tanaka has received honoraria from Bristol-Myers Squibb and Gilead Sciences, and has received commercial research funding from Chugai, AbbVie, Bristol-Myers Squibb, Janssen, and Gilead. Masayuki Kurosaki has served in an advisory role to AbbVie, Gilead, GlaxoSmithKline, and Otsuka, and has received honoraria from AbbVie, Bristol-Myers Squibb, Chugai, Daiichi Sankyo, Gilead, GlaxoSmithKline, Janssen, Merck Sharp & Dohme, Otsuka, and Toray. Fusao Ikeda, Tomohide Tatsumi, Yasuhiro Takikawa, and Eiji Mita, declare no conflicts of interest.

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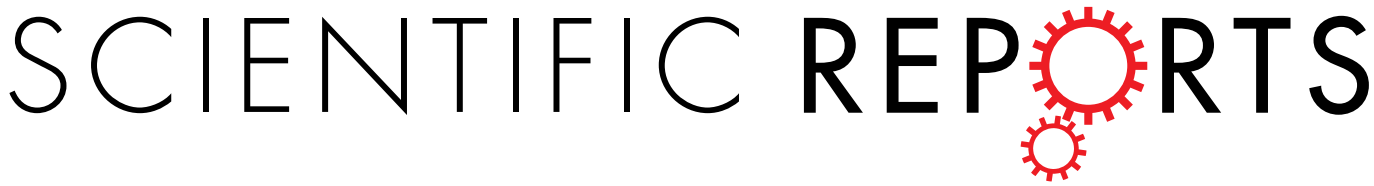

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Genome-wide association study identified new susceptible genetic variants in HLA class I region for hepatitis B virus-related hepatocellular carcinoma

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We have performed a genome-wide association study (GWAS) including 473 Japanese HBV (hepatitis B virus)-positive HCC (hepatocellular carcinoma) patients and 516 HBV carriers including chronic hepatitis and asymptomatic carrier individuals to identify new host genetic factors associated with HBV-derived HCC in Japanese and other East Asian populations. We identified 65 SNPs with P values $< 10^{-4}$ located within the HLA class I region and three SNPs were genotyped in three independent population-based replication sets. Meta-analysis confirmed the association of the three SNPs (rs2523961: OR = 1.73, $P = 7.50 \times 10^{-12}$; rs1110446: OR = 1.79, $P = 1.66 \times 10^{-13}$; and rs3094137: OR = 1.73, $P = 7.09 \times 10^{-9}$). We then performed two-field HLA genotype imputation for six HLA loci using genotyping data to

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investigate the association between HLA alleles and HCC. HLA allele association testing revealed that *HLA-A*33:03* (OR = 1.97, P = 4.58 × 10^{-4}) was significantly associated with disease progression to HCC. Conditioning analysis of each of the three SNPs on the HLA class I region abolished the association of *HLA-A*33:03* with disease progression to HCC. However, conditioning the HLA allele could not eliminate the association of the three SNPs, suggesting that additional genetic factors may exist in the HLA class I region.

Hepatitis B (HB) is a potentially life-threatening liver infection caused by hepatitis B virus (HBV), and approximately 248 million people worldwide are estimated to be chronically infected with HBV[1]. The clinical course of HBV infection is variable, including acute self-limiting infection, fulminant hepatic failure, inactive carrier state, and chronic hepatitis with progression to liver cirrhosis and hepatocellular carcinoma (HCC). Although some HBV carriers spontaneously eliminate the virus, every year 2–10% of individuals with chronic HB (CHB) develop liver cirrhosis, and a subset of these individuals suffer from liver failure or HCC[2]. Around 600,000 new HCC cases are diagnosed annually worldwide, and it is relatively common in Asia-Pacific countries and sub-Saharan Africa. More than 70% of HCC patients are diagnosed in Asia[3]. In contrast, HCC is relatively uncommon in the USA, Australia, and European countries[3,4]. The majority of HCC cases develop in patients with cirrhosis, which is most often attributable to chronic HBV infection followed by chronic hepatitis C virus infection in the Asia-Pacific region[5].

Human leucocyte antigen (HLA) proteins present self and non-self peptides to T cell receptors (TCRs) to maintain self-tolerance and adapted immunity. The HLA region resides on the short arm of chromosome 6, designated as 6p21.3. It is about 3.6 Mb in length and more than 200 functional and nonfunctional genes[6,7] are located in the region. The whole HLA region is divided into three subgroups, which are designated as class I, II, and III. The HLA class I region contains 19 HLA class I genes including 3 classical (*HLA-A*, *-B*, and *-C*), 3 non-classical (*HLA-E*, *-F*, and *-G*), and 12 non-coding genes or pseudogenes. The HLA class II region contains classical class II alpha- and beta-chain genes of *HLA-DR*, *-DQ*, and *-DP*. All HLA class I and class II molecules can present peptides to T cells, but each protein binds a different range of peptides. The presence of several different genes of each HLA class means that any one individual is equipped to present a much broader range of peptides than if only one HLA molecule of each class were expressed at the cell surface. A total of 17,695 *HLA* alleles (12,893 in class I and 4,802 in class II) were released by The IPD-IMGT/HLA database release 3.31.0 in January 2018 (https://www.ebi.ac.uk/ipd/imgt/hla/). Of the 12,893 class I alleles, 4,181, 4,950, and 3,685 alleles were registered in *HLA-A*, *-B*, and *-C* genes, respectively. Of 4,802 class II alleles, 2,146, 1,178, and 965 alleles were registered in *HLA-DRB1*, *-DQB1*, and *-DPB1* genes, respectively.

Recent genome-wide association studies (GWAS) of chronic HBV carriers with or without HCC in Chinese populations reported that one SNP (rs17401966) in *KIF1B*, two SNPs (rs9272105 and rs455804) in *HLA-DQA1/DRB1* and *GRIK1*, and two SNPs (rs7574865 and rs9275319) in *STAT4* and *HLA-DQ* were associated with disease progression to HCC[8–10]. A number of candidate genes have been investigated by genetic association studies to evaluate their roles in susceptibility to HCC. The findings from these studies, however, are inconclusive due to insufficient evidence and a lack of independent validation. All three papers referred to in this manuscript performed GWAS and replication studies using only Chinese population samples. For example, the study by Zhang *et al.*[10] used 2,310 cases and 1,789 controls of Chinese ancestry and identified one intronic SNP in *KIF1B* associated with HBV-related HCC. This result, however, was not replicated in several other populations[11,12]). These findings suggest that GWAS and subsequent replication studies should be conducted in populations other than Chinese.

In this study, we performed GWAS using Japanese CHB patients with and without HCC and a replication study using East Asian populations including Japanese, Hong Kong Chinese, and Thai.

Results

GWAS and replication study of HBV-related HCC. We conducted a GWAS using samples from 473 Japanese HBV-positive HCC patients and 516 HBV carriers including CHB and asymptomatic carrier (ASC) individuals by analyzing 447,830 autosomal SNPs. Figure 1 shows a genome-wide view of the SNP association data based on allele frequencies. There were 110 SNPs with P values < 10^{-4} in the GWAS (Supplementary Materials, Table S1). Of the 110 SNPs, 65 and 4 SNPs were located on the HLA class I and II regions, respectively. These results suggested that HBV-related HCC could be associated with SNPs located in the HLA region, although associations did not reach the genome-wide significance level. Outside the HLA region, there were 41 SNPs with P values < 10^{-4} and 4 SNPs showed P values < 10^{-5}.

In order to validate these suggestive associations, we selected seven SNPs based on the following criteria: P values < 10^{-4} in the HLA region and < 10^{-5} outside the HLA region and only SNPs with the lowest P value or highest OR were selected when multiple SNPs showed strong LD. Three independent sets of HBV-related HCC cases, CHB and ASC controls (replication-1: Japanese 153 cases and 614 controls; replication-2: Hong Kong Chinese 94 cases and 187 controls; and replication-3: Thai 185 cases and 198 controls), and the original GWAS set of 989 Japanese samples (473 cases and 516 controls) were genotyped and used in a subsequent replication analysis. Of the seven SNPs, four (rs2523961, rs1110446, and rs3094137 located on HLA class I region, and rs2295119 located on HLA class II region) were validated, and consistent associations were observed between the original GWAS set and replication sets (Table 1). For these four SNPs, no heterogeneity of association was observed between the original GWAS samples and the replication samples. Two SNPs in the HLA region (rs2523961 and rs1110446) showed a genome-wide significant association (rs2523961: OR = 1.91, P = 6.42 × 10^{-10}; and rs1110446: OR = 1.93, P = 2.52 × 10^{-10}) using the combined Japanese samples (GWAS and replication-1) (Table 1). Moreover, the meta-analysis with the combined Japanese samples and two independent sample sets (Hong Kong Chinese and

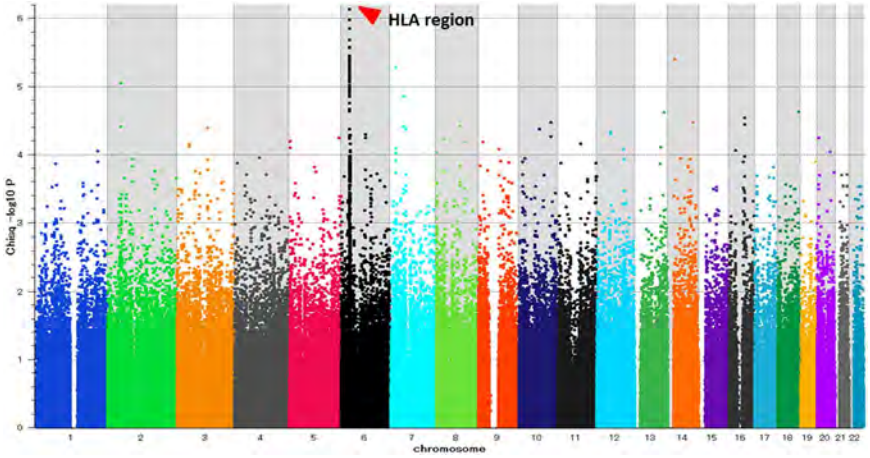

Figure 1. GWAS result. GWAS included 989 samples [473 Japanese HCC cases and 516 Japanese HBV carrier (CH and ASC) controls]. P-values were calculated using the chi-square test for allele frequencies among 447,830 SNPs.

Thai) confirmed associations for the two SNPs (rs2523961: $P = 5.81 \times 10^{-11}$; and rs1110446: $P = 9.09 \times 10^{-13}$), while the remaining two SNPs showed a marginal association (rs3094137: OR = 1.76, $P = 3.91 \times 10^{-7}$; and rs2295119: OR = 0.63, $P = 5.51 \times 10^{-7}$).

Association test for imputed HLA alleles. The two SNPs showing genome-wide significant associations were located on HLA class I region, and the marginally associated SNP was located on HLA class I and II region. To investigate the association of *HLA* alleles, we performed two-field HLA genotype imputation for six HLA loci (*HLA-A, -B, -C, -DRB1, -DQB1*, and *-DPB1*) using 989 genome-wide genotyping data used for the GWAS. Imputed *HLA* alleles were filtered (Call Threshold < 0.5) before performing association analysis for each HLA locus. The results of association tests in *HLA-A, -B, -C, -DRB1, -DQB1*, and *-DPB1* alleles are shown in Table 2 and Supplementary Materials, Table S2. To avoid false-positive results due to multiple testing for 77 HLA alleles, significance levels were set at 0.000649 (=0.05/77). A protective effect of *HLA-DPB1*02:01* (OR = 0.59, $P = 5.23 \times 10^{-6}$) was observed as previously reported[13]. We also detected that *HLA-A*33:03* was significantly associated with disease progression to HCC (OR = 1.97, $P = 4.58 \times 10^{-4}$) (Table 2).

Using GTEx-generated eQTL data[14], we checked for correlations between the three SNPs and *HLA-A* gene expression levels. The SNP rs2523961 was correlated with *HLA-A* gene expression in various tissues (muscle: $P = 6.1 \times 10^{-20}$; heart: $P = 2.3 \times 10^{-15}$, 2.1×10^{-11}; esophagus: $P = 2.8 \times 10^{-12}$, 1.8×10^{-6}; artery: $P = 4.7 \times 10^{-12}$, 3.9×10^{-11}; thyroid: $P = 1.4 \times 10^{-11}$; pancreas: $P = 3.3 \times 10^{-9}$; brain: $P = 1.9 \times 10^{-8}$, 2.2×10^{-7}; nerve: $P = 3.2 \times 10^{-8}$; testis: $P = 5.5 \times 10^{-7}$; lung: $P = 1.7 \times 10^{-5}$). The SNP rs1110446 was also associated with *HLA-A* gene expression in muscle ($P = 5.5 \times 10^{-15}$), skin ($P = 6.2 \times 10^{-11}$, 4.4×10^{-9}), artery ($P = 8.7 \times 10^{-6}$, 1.1×10^{-4}), esophagus ($P = 2.5 \times 10^{-5}$), and whole blood ($P = 5.1 \times 10^{-5}$). These results suggest that these SNPs affected *HLA-A* gene expression.

Conditioning each of the three SNPs on the HLA class I region (Supplementary Material, Fig. S1a–c) abolished the association of *HLA-A*33:03* ($P > 0.05$), but conditioning of *A*33:03* could not eliminate the association of the three SNPs (rs2523961: OR = 1.69, $P = 7.06 \times 10^{-4}$; rs1110446: OR = 1.65, $P = 9.33 \times 10^{-4}$; and rs3094137: OR = 1.54, $P = 5.68 \times 10^{-3}$) (Fig. 2). These conditional analyses suggest that additional genetic factors other than *HLA-A* allele exist in the HLA class I region. In contrast to the class I region, conditional analysis controlling for the SNP rs2295119 using *DPB1*02:01* allele suggests that *DPB1* allele could abolish the association of rs2295119 on the HLA class II region ($P > 0.05$) (Supplementary Material, Fig. S1e).

Discussion

In the current GWAS, we found a marginal association between an SNP (rs2295119) located in the *HLA-DPB* region and HBV-related HCC. Moreover, the association analysis of *HLA-DPB1* alleles and the conditional analysis with *HLA-DPB1*02:01* suggested that *DPB1*02:01* was the major protective allele in the HLA class II region. Recent GWAS also showed that SNPs located in the HLA class II region (*HLA-DQA1/DRB1*[9] and *HLA-DQ*[8]) were associated with HBV-related HCC in the Chinese population. We focused on the p-values of the HLA class II region (*HLA-DQ* and *-DR*) and six other gene regions (*KIF1B, UBE4B, PGD*, 8p12, *GRIK1* and *STAT4*) reported in previous studies and revealed the SNPs of four regions (*HLA-DQ* and *-DR*, 8p12, and *STAT4*) had p-values of less than 0.00625 (0.05/8). There were 52, 10 and 1 SNP with $P < 0.00625$ located on *HLA-DQ/DR*, 8p12, and *STAT4*, respectively, and the lowest p-value of each region was 0.00102 (rs9271894 on *HLA-DQA1*, OR = 1.46), 0.00278 (rs8084 on *HLA-DRA*, OR = 1.32), 0.00049 (rs13250548 on 8p12, OR = 0.68), and 0.0019 (rs6752770 on *STAT4*, OR = 1.44).

We also identified significant associations in the HLA class I region, especially around the *HLA-A* locus. The association test of imputed HLA alleles and conditional analyses with *HLA-A*33:03* suggested that *HLA-A*33:03* is the susceptibility allele for HCC. We performed additional conditional analyses controlling for the SNP on chromosome 6 using *A*33:03* and *DPB1*02:01* alleles. This indicated that *HLA-A* and *DPB1* alleles could

Marker	Allele (1/2)	stage	population	cases				controls				P value[b]	OR (95% CI)
				11	12	22	MAF	11	12	22	MAF		
rs2523961	A/G	GWAS	Japanese	12	174	287	0.209	11	111	394	0.129	2.57E-07	2.02 (1.54–2.66)
(class I)		Combined	Japanese	19	219	388	0.205	23	238	867	0.126	6.42E-10	1.91 (1.56–2.37)
		Replication2	Hong Kong Chinese	1	25	68	0.144	2	34	151	0.102	0.118	1.55 (0.90–2.66)
		Replication3	Thai	13	54	108	0.229	6	49	142	0.155	0.059	1.49 (0.98–2.28)
		Meta-analysis[a]										5.81E-11	
rs1110446	T/C	GWAS	Japanese	14	177	282	0.217	11	114	391	0.132	4.44E-08	2.10 (1.60–2.75)
(class I)		Combined	Japanese	21	222	383	0.211	24	245	861	0.130	2.52E-10	1.93 (1.57–2.37)
		Replication2	Hong Kong Chinese	2	22	70	0.138	1	35	151	0.099	0.138	1.52 (0.90–2.62)
		Replication3	Thai	14	66	100	0.261	5	51	142	0.154	0.002	1.93 (1.27–2.92)
		Meta-analysis[a]										9.09E-13	
rs3094137	A/G	GWAS	Japanese	9	150	314	0.178	10	97	409	0.113	9.65E-05	1.74 (1.31–2.31)
(class I)		Combined	Japanese	13	191	421	0.174	19	203	906	0.107	3.91E-07	1.76 (1.41–2.19)
		Replication2	Hong Kong Chinese	0	8	86	0.043	0	9	178	0.024	0.201	1.93 (0.71–5.21)
		Replication3	Thai	0	19	160	0.053	0	15	181	0.038	0.468	1.35 (0.60–3.03)
		Meta-analysis[a]										9.83E-05	
rs2295119	T/G	GWAS	Japanese	18	139	316	0.185	41	191	284	0.265	5.77E-06	0.59 (0.47–0.74)
(class II)		Combined	Japanese	27	179	420	0.186	78	417	635	0.254	5.51E-07	0.63 (0.53–0.76)
		Replication2	Hong Kong Chinese	2	22	70	0.138	5	54	128	0.171	0.318432	0.78 (0.47–1.28)
		Replication3	Thai	4	39	136	0.131	3	50	143	0.143	0.285443	0.76 (0.47–1.25)
		Meta-analysis[a]										4.88E-07	

Table 1. Four SNPs in the HLA region associated with disease progression to HCC. [a]Results of meta-analysis were calculated by the DerSimonian-Laird method. [b]Result of logistic regression analysis adjusted for age and sex.

abolish the association in the HLA class II region but were not sufficient to abolish the association in the HLA class I region (Fig. 2 and Supplementary Material, Fig. S1f). Therefore, not only the *HLA-A* allele but also additional genetic factor(s) likely exist in the HLA class I region. There are several genes in this region including *HLA-A*, *HCG9, HLA-J, HCG8, ZNRD1-AS1, ZNRD1, PPP1R11, RNF39, TRIM31*, and *TRIM40* (shown in Fig. 2). Although these genes include pseudogenes and poorly characterized genes, some are associated with various diseases. The zinc ribbon domain-containing 1 (ZNRD1) protein is associated with cell growth of gastric cancer cells[15], angiogenesis of leukemia cells[16], and HIV-1/AIDS disease progression[17,18]. In addition, *ZNRD1* knockdown inhibits the expression of HBV mRNA and promotes the proliferation of HepG2.2.15 cells[19], suggesting that *ZNRD1* is one of the possible additional genetic factors at the HLA class I region. The tripartite motif-containing 31 (TRIM31) protein is essential for promoting lipopolysaccharide-induced Atg5/Atg7-independent autophagy[20]. Moreover, *TRIM40* is downregulated in gastrointestinal carcinomas and chronic inflammatory lesions of the gastrointestinal tract[21].

Non-self antigens, such as virus-infected cells and cancer cells, and HLA class I molecules are generally recognized by the TCRs on CD8+ T lymphocytes, resulting in T cell activation[22]. The activated T cells divide and some of their progeny differentiate into lymphocytes capable of killing cells (cytotoxic T lymphocytes: CTLs) displaying the same peptides (such as tumor-specific peptides) on their HLA class I molecules. These CTLs target tumor-specific antigenic peptides and eliminate them. In other words, CTLs cannot eliminate cancer cells without HLA class I molecules even if the person has tumor-specific peptides. Cancer cells therefore need to escape from the immune system for patients to be identified as having cancer.

In this study, we identified a significant association between *HLA-A*33:03* and HBV-related HCC. In addition to *HLA-A*33:03*, previous studies and this study suggested that *HLA-DR*, *-DQ*, and *-DP* were associated with disease progression[8,9,13]. Functional analysis of HLA class I and II proteins could be an important step in determining the pathology of HBV-related HCC.

HLA-A	Case (2n = 892)	%	Control (2n = 998)	%	Fisher's P-value	OR	95% CI
02:01	105	11.8	113	11.3	0.7733	1.04	0.78–1.40
02:06	80	9.0	106	10.6	0.2462	0.83	0.60–1.14
02:07	38	4.3	40	4.0	0.8174	1.07	0.66–1.72
11:01	53	5.9	94	9.4	0.005757	0.61	0.42–0.87
24:02	331	37.1	393	39.4	0.3198	0.91	0.75–1.10
26:01	72	8.1	89	8.9	0.5636	0.90	0.64–1.26
26:03	18	2.0	22	2.2	0.8732	0.91	0.46–1.80
31:01	112	12.6	90	9.0	0.01384	1.45	1.07–1.97
33:03	76	8.5	45	4.5	**0.00046**	**1.97**	1.33–2.95

Table 2. Association analyses of *HLA-A* alleles.

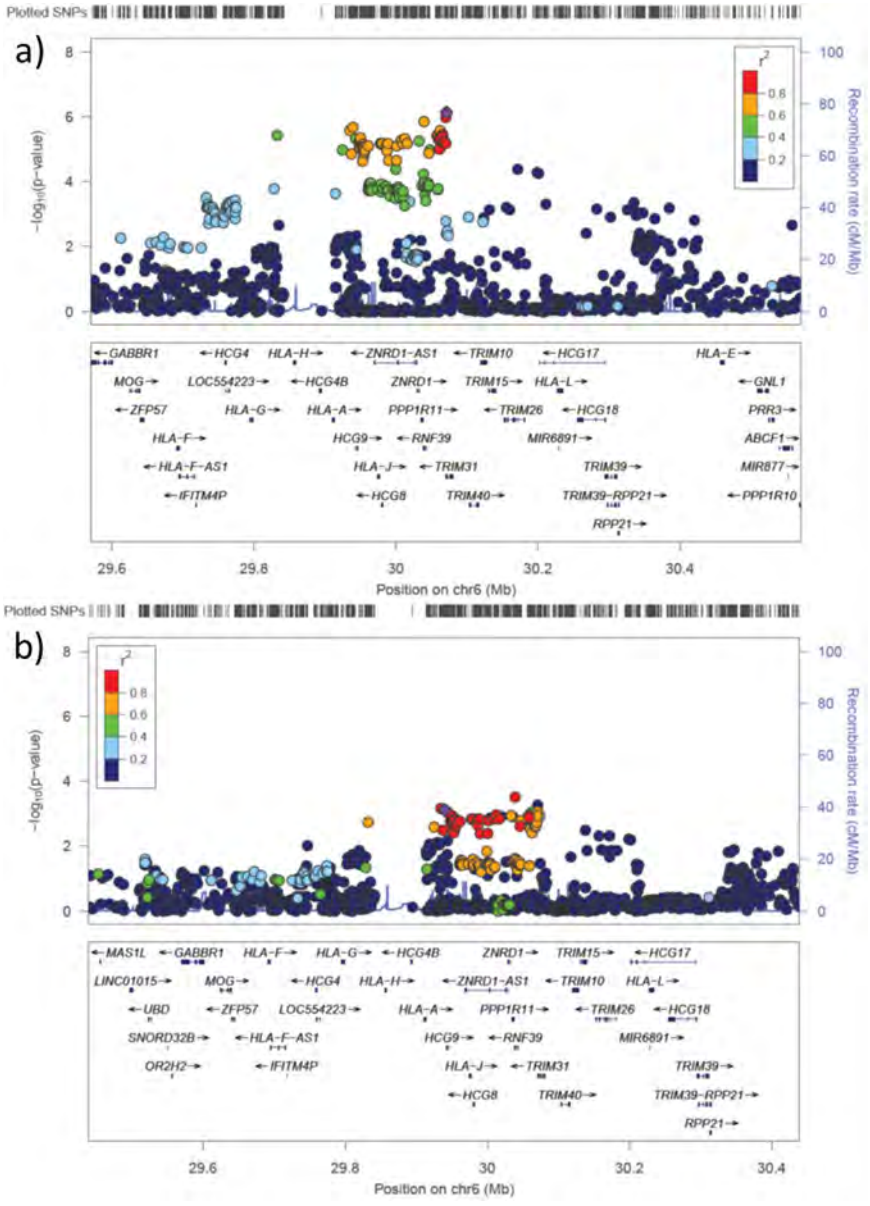

Figure 2. Association plots of the HLA class I region on chromosome 6 HLA region. (**a**) The major genetic determinant of HBV-related HCC risk to HLA class I genes. (**b**) Conditional analysis controlling for the effect of *HLA-A*33:03*.

Methods

Ethics statement. All study protocols conformed to the relevant ethical guidelines, as reflected in the *a priori* approval by the ethics committee of the University of Tokyo, and by the ethics committees of all participating universities and hospitals. All participating studies obtained informed consent from all participants in this study and all samples were anonymized.

Samples. Samples from 3,133 individuals who had HBV-derived chronic hepatitis, ASC, liver cirrhosis, or HCC and patients with other HBV-related symptoms were collected by 26 universities and hospitals (Hokkaido University Hospital, Teine Keijinkai Hospital, Iwate Medical University Hospital, Musashino Red Cross Hospital, The University of Tokyo Hospital, Saitama Medical University Hospital, Chiba University Hospital, Kitasato University Hospital, Kohnodai Hospital, Shinshu University Hospital, Kanazawa University Hospital, Nagoya City University Hospital, Kyoto Prefectural University of Medicine Hospital, National Hospital Organization Osaka National Hospital, Osaka City University Hospital, Hyogo College of Medicine, Tottori University Hospital, Ehime University Hospital, Yamaguchi University Hospital, Kawasaki Medical College Hospital, Okayama University Hospital, Nagasaki Medical Center, Kurume University Hospital, Saga University Hospital, Eguchi Hospital, and Kyusyu University Hospital). The Japanese Public Health Cancer-based Prospective (JPHC) Study samples[23] in Japan were used for the replication study. Hong Kong Chinese samples were collected at the University of Hong Kong. Thai samples were collected at Chulalongkorn University.

HBV status was measured based on serological results for HBsAg and anti-HBc with a fully automated chemiluminescent enzyme immunoassay system (Abbott ARCHITECT, Abbott Japan, Tokyo, Japan or LUMIPULSE G1200, Fujirebio, Inc., Tokyo, Japan). For clinical staging, ASC state was defined by the presence of HBsAg with normal ALT levels over 1 year (examined at least four times at 3-month intervals) and without evidence of liver cirrhosis. CH was defined by elevated ALT levels (1.5 times the upper limit of normal [35 IU/L]) persisting for over 6 months (by at least three bimonthly tests). HCC was diagnosed by ultrasonography, computerized tomography, magnetic resonance imaging, angiography, tumor biopsy, or by a combination of these.

SNP genotyping and data cleaning. For the GWAS, we genotyped 1,356 Japanese samples using the Affymetrix Axiom Genome-Wide ASI 1 Array (Affymetrix, Inc., Santa Clara, CA, USA) according to the manufacturer's instructions and determined the genotype calls of 600,307 SNPs using the Genotyping Console v4.2.0.26 software (Supplementary Material, Fig. S2a). To increase the samples for genotyping, we used not only CHB patients with and without HCC but also patients with HBV-related other symptoms such as liver cirrhosis. All samples used for genotyping passed a Dish QC >0.82 and overall call rate $>97\%$. The average Dish QC for 1,356 samples was 0.969 (0.883–0.993) and the average call rate reached 99.42% (97.47–99.87%). All genotyped samples passed a heterozygosity check, and 25 duplicated samples were identified in identity by descent (IBD) testing. A principal component analysis (PCA) found seven outliers could be excluded by the Smirnov-Grubbs test, and we showed that all the remaining samples ($n = 1,324$) formed a single cluster with the HapMap Japanese (JPT) samples but not with the Han Chinese (CHB), Northern and Western European (CEU), and Yoruban (YRI) samples. We then applied the following thresholds for SNP quality control in data cleaning: SNP call rate of $\geq 95\%$, minor allele frequency of $\geq 3\%$ and Hardy-Weinberg equilibrium P value of ≥ 0.001. A total of 447,830 SNPs on autosomal chromosomes passed the quality control filters and were used for subsequent GWAS. For the association study of HBV-related HCC, we selected 481 HBV-related HCC patients (cases) and 538 HBV carriers (CH and ASC patients, controls) from 1,324 samples and performed IBD testing and PCA again for these samples. Twenty-three related samples and seven outliers were excluded by IBD testing and PCA (Supplementary Material, Fig. S3), respectively. We finally used 473 cases and 516 controls for GWAS. A quantile-quantile plot of the distribution of test statistics for the comparison of genotype frequencies in the cases and controls showed that the inflation factor λ was 1.016 for all tested SNPs and was 1.009 when SNPs in the HLA region were excluded (Supplementary Material, Fig. S4). All cluster plots for SNPs with P values of $<10^{-4}$ were checked visually and SNPs with ambiguous genotype calls were excluded.

In the replication stage, we selected seven SNPs with P values of $<10^{-5}$ from the results of the chi-square test in the GWAS. A TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) was used to confirm the genotypes at each SNP. We genotyped 989 and 767 Japanese samples for the validation of the GWAS and for the replication study, respectively. We further genotyped 281 Hong Kong Chinese and 383 Thai samples for the replication study (Supplementary Materials, Table S3).

Statistical analysis. The characteristics of analyzed samples are shown in Supplementary Materials, Table S3. For the GWAS and replication study, the chi-square test was applied to a two-by-two contingency table in the allele frequency model. Meta-analysis was performed using the DerSimonian-Laird method (random-effects model) in order to calculate the pooled OR and its 95% confidence interval. Fisher's exact test in a two-by-two contingency table was used to examine the association between *HLA* alleles and disease progression of HBV patients. To avoid false-positive results due to multiple testing, the resulting P-values were adjusted based on the number of observed alleles with frequencies $\geq 0.5\%$ in cases and controls. Conditional logistic regression analysis was performed for SNPs and *HLA* alleles. This analysis was performed as implemented in Plink v1.07 software[24], conditioning on *HLA-A*33:03* and *DPB1*02:01* to each of the other SNPs. Other statistical analyses were performed using the SNP & Variation Suite 7 software (Golden Helix, Bozeman, MT, USA) and statistical software R v2.6. Manhattan plot of conditioning of each SNP or HLA allele was generated by LocusZoom[25].

HLA imputation. SNP data from 989 samples were extracted from extended MHC (xMHC) regions ranging from 25759242 bp to 33534827 bp based on hg19 position. Two-field HLA genotype imputation was performed for a total of six HLA class I and class II genes using the HIBAG R package[26,27]. For *HLA-A,-B, -DRB1, -DQB1*,

and -*DPB1*, a Japanese imputation reference[26] was used for HLA genotype imputation. For *HLA-C*, the HIBAG Asian reference[27] was used for HLA genotype imputation. We applied post-imputation quality control using call-threshold (CT > 0.5); the call rate of successfully imputed samples ranged from 88.7 to 98.5% for the six HLA classes. In total, we imputed 5,650 HLA genotypes in HLA class I and class II genes.

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Study desingn and discussion: H.S., N.N., M.H., M.S., N. Sw., S.T., K.K., Y.K., H.Y., S. Ng., A. Tk., M.F., M.K., I.N., J.-H.K., K.M., K.H., S. Ns., A.M., E.T., N. Sk., K.O., K. Ymm., A. Tm., O.Y., T.K. I.S., Y.I., Y.E., S.O., S.M., M.-F.Y.,

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a p value<0.001. The factors associated with bullying were the younger age group, shorter length of service, shifting work, non-managerial position and the designation as a doctor.

Conclusion A significant proportion of healthcare workers had been bullied, and bullying exposure was shown to be associated with depression and low self-esteem. Hence, regular screening for bullying, depression and low self-esteem should be done to enable early intervention.

1551 CHANGES IN TWENTY YEARS OF THE EPIDEMIOLOGICAL STATUS OF NEEDLESTICK/SHARPS INJURIES REPORTED TO JAPAN-EPINET THROUGH A NATION-WIDE SURVEILLANCE NETWORK

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Introduction This study aimed at examining annual logs of needlestick/sharps injuries (NSIs) collected through a voluntary nation-wide surveillance network in twenty-years for preventing occupational blood-borne infections. The emphasis was placed on revealing the past and current situations of NSIs in health care settings.

Methods Japan-EPINet format was developed by the technical support of the International Healthcare Worker Safety Centre, University of Virginia in the United States in 1996. Japan-EPINet Surveillance (JES) was conducted by the Research Group for Occupational Infection Control and Prevention in Japan (JRGOICP). Data were analysed in four phases of the nation-wide surveillance network of AIDS referral hospitals out of a total of 364 registered, a total number of hospital-year was 1879. These hospitals reported employees' percutaneous injuries on a voluntary basis.

Results A total of 65,032 NSIs were reported to Japan-EPINet from 1996 to 2015. The rate of hepatitis C antibody positive cases of the total NSIs decreased from 69.9% (1,511/2,161) in 1996 to 11.5% (714/6,201) in JES2015. The proportion of NSIs due to 'recapping' decreased (28.7%, 6.9% respectively). Devices caused to NSIs by winged steel needles (25.3%, 8.6%) and vacuum tube phlebotomy needles (4.8%, 1.7%) were decreased, disposal syringe (28.5%, 26.2%) and IV catheter (6.7%, 5.2%) were fairly decreased. The proportion of Suture needle (10.3%, 16.9%) and pre-filled cartridge syringe (2.8%, 8.3%) were increased.

Discussion The changes of characteristics NSIs in Japan in twenty-year suggested that recognition of the risks of NSIs was vital for promoting the effective use of safety-engineered needle/sharp devices and point-of-use disposal containers because the rate of hepatitis C antibody positive cases among voluntary reported NSIs. The creation of the nation-wide surveillance network was effective for monitoring and evaluating NSIs and for focusing on implementation of effective countermeasures.

25 PREPARATION OF HAZARDOUS DRUGS IN BIOLOGICAL SAFETY CABIN (BSC): THE CHALLENGE OF GETTING HEALTHIER WORK ENVIRONMENTS

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Introduction Hazardous drugs are an important risk to health care workers. Some of these products may even be potentially carcinogenic.

In different Spanish hospitals it was observed that only Cytostatics drugs were prepared in biological safety cabins, leaving workers exposed to the rest of hazardous non cytostatic drugs.

Methods A bibliographical review of scientific articles and researches has been carried out, together with the laws on occupational health and recommendations of the Spanish organisms.

In the USA, research promoted the development of policies of prevention and the incorporation of these drugs in the list NIOSH.

Result After analysing the information obtained, we detected the following problems: HD's are prepared in hospitalisation rooms, where the right conditions to protect workers are non-existent; In many cases, health care workers are given only personal protective equipment to avoid exposure; Specific health control isn't performed in most cases; National legislation obliges the risk to be taken into account for the worker. Although there are no long-term epidemiological studies, protective measures should be taken.

Discussion In many hospitals in our country HD's are not prepared in biological safety cabins. Health workers are unaware that they are exposed to these risks and no specific health training or monitoring is performed. Collaborative epidemiological researches should be promoted among Public Health Units, which have information on the prevalence rate of cancer diseases, and those responsible for occupational health prevention.

250 HOW THE WORKING BACKS PROGRAMME HELPED STAFF MANAGE BACK PAIN, REMAIN IN WORK AND REDUCE ABSENTEEISM

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Introduction The Working Backs Programme (WBP) is designed for staff reporting back pain as a result of work or whose work performance is affected. It's a comprehensive approach including medical assessment, provision of information and education, a designated physiotherapy and ergonomic staff referral service and a referral pathway for further investigations and/or review. The effectiveness was evaluated by an initial audit in 2012 and subsequent audits in 2015 and 2016.

Methods Data was collected through questionnaires at initial consultation and post discharge for comparison. This included

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＜特別寄稿＞

日本肝臓学会評議員を対象としたB型肝炎ワクチンに関するアンケート調査

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要旨：B型肝炎（HB）ワクチンの在り方を検討するために，日本肝臓学会HBワクチンワーキンググループとして日本肝臓学会評議員などを対象にHBワクチンに関するアンケート調査を実施した．その結果，1)「HBワクチンの適切な接種時期（キャッチアップ）」に関しては，小学生高学年64%と最多であった．2)「ワクチン無効例に対する対策」としては，筋肉内注射や4回以上投与などが挙げられた．3)「HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性」について，「必要」が63%で最も多く，その施設の多くは職員に対するHBs抗体の定期検査を12カ月ごとに行い，HBs抗体価10 mIU/mL未満の時点でHBワクチンを追加接種していた．これらの結果を踏まえると，「追加のワクチン接種は必要ではない」とする日本環境感染学会ガイドラインについて再度議論する必要があるように思われた．

索引用語：　HBV　B型肝炎ワクチン　ワクチンブースター　HBs抗体

緒　言

わが国では，1972年に日本赤十字社の血液センターにおけるHBs抗原のスクリーニング検査が開始された．さらに，1986年に開始された母子感染防止事業に基づく出生児に対するワクチンおよび免疫グロブリン投与により，垂直感染による新たなHBVキャリア成立が阻止され，若年者におけるHBs抗原陽性率は著しく減少した．しかし，一方で性交渉に伴う水平感染によるB型急性肝炎の発症数は減少せず，近年では，肝炎が遷延し慢性化しやすいゲノタイプAのHBV感染が増加傾向にある[1)]．

2016年10月より0歳児を対象としたB型肝炎（HB）ワクチンの定期接種が開始されたが，定期接種の対象から漏れた小児への対応，性行為感染症としてのB型急性肝炎，ワクチン無反応・低反応者対策，ブースター接種の必要性，HBワクチン接種によるHBV再活性化抑制などの問題が残されている．

また，HBVワクチン接種によって免疫が得られても，HBs抗体は最初の1年で急速に低下し，それ以降はゆっくりと減少する．健常人では，ワクチン接種者の90～95%に抗体産生がみられるが，抗体産生は時間の経過とともに減弱し，8年以上経過すると約60%の人で抗体が検出されなくなる．しかし，HBVに対する免疫は保たれるため，再度ワクチンを接種する必要はないとしている[2)3)]．実際，4～23年前にワクチンが接種されてHBs抗体を獲得したにも拘わらず，時間の経過によって10 mIU/mL未満まで低下してしまった人にワクチンをブースター接種すると僅か2～4週間後に74～100%の人で抗体が再陽転化した．このデータはワクチン接種者の多くが免疫記憶を維持しており，HBVの曝露によってHBs抗体を獲得することができることを示している．以上の結果を踏まえて，米国CDC（Centers for Disease Control and Prevention）ガイドラインでは，一度十分な抗体価が得られれば，その後抗体価が低下しても曝露に際して効果的な免疫反応が得られると判断され，腎不全を含む免疫不全症例以外は，経時的な抗体価測定は不要とした[4)]．

今回，HBワクチンの在り方を検討するために，小池和彦理事長の承認の下，企画広報委員会（持田　智委員長）に依頼して，同委員会内にHBワクチン小委員会を設置し，日本肝臓学会HBワクチンワーキンググループ（WG）として日本肝臓学会評議員などを対象にHBワクチンに関するアンケート調査を実施したので，その結果を報告する．

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Table 1　B型肝炎ワクチンに関するアンケートの様式

B型肝炎ワクチンに関するアンケートのお願い

一般社団法人　日本肝臓学会
企画広報委員会　委員長　持田智
HBワクチン小委員会

2016年10月より0歳児を対象としたB型肝炎(HB)ワクチンの定期接種が開始されました。現在残された問題点として、定期接種の対象から漏れた小児への対応、性行為感染症としてのB型急性肝炎（欧米型A）及びHBV再活性化があり、これらの点に関して学会として対応を考えるべく、「HBワクチン小委員会」が発足致しました。つきましては今回、日本肝臓学会評議員の先生方のご意見を伺いたく簡単なアンケートを実施させて頂きますので、以下の質問に対する御回答をお願いします。**いずれも複数回答可です。**

1．定期接種の対象とならなかった人に対するキャッチアップとしてHBワクチンの適切な時期についてお尋ねします。
　□ 小学生高学年（他のワクチンと同時接種）
　□ 中学生　　□ 高校生
　□ キャッチアップ必要なし

2．ワクチン無効例に対する対策はどのようにされていますか？これまでの報告（八橋弘 B型肝炎ワクチンの筋肉内注射. 日本医事新報 4858:53-58, 2012）によると筋肉内注射により有意なHBs抗体価上昇が期待できます。
　（接種方法の変更）□ 筋肉内注射　　□ 皮内注射
　□ ワクチンの種類を変更　　□ 倍量投与　　□ 4回以上投与
　□ その他（　　）

3．院内で、職員に対するHBs抗体の採血は定期的にされていますか？
　□ はい　　□ いいえ
　「はい」の場合の頻度（　　）ヶ月おき

4．HBs抗体価が低下した医療従事者に対するHBワクチンのブースターはされていますか？　　□ はい　　□ いいえ
　「はい」の場合の目安
　□ HBs抗体 10 mIU/mL未満（陰性）　　□ HBs抗体100 mIU/未満

5．その他、ご意見がございしましたら、よろしくお願いします。

方　法

平成29年9月, 日本肝臓学会HBワクチンワーキンググループとして日本肝臓学会評議員など855名を対象にTable 1のようなアンケート調査を実施した. 1)定期接種の対象とならなかった人に対するキャッチアップとしてHBワクチンの適切な接種時期, 2)ワクチン無効例に対する対策, 3)院内職員に対するHBs抗体の定期検査の実施状況, 4)HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性と実際の対応について質問した.

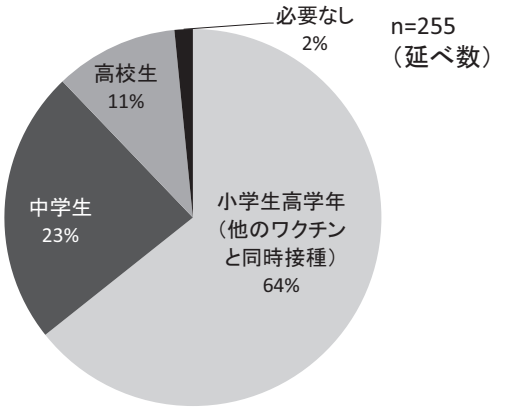

Fig. 1　HBワクチンの適切な接種時期（キャッチアップ）

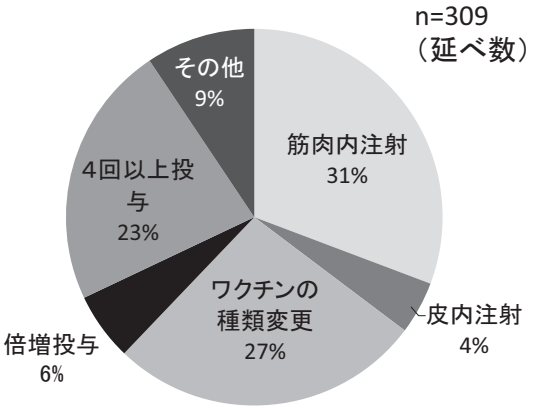

Fig. 2　ワクチン無効例対策

結　果

アンケート調査の回収率は24%（206/805）であった．1）「HBワクチンの適切な接種時期（キャッチアップ）」に関しては，小学生高学年（他のワクチンと同時接種）64%，中学生23%，高校生11%であった（Fig. 1）．2）「ワクチン無効例に対する対策」としては，筋肉内注射31%（皮内注射4%），ワクチンの種類を変更27%，4回以上投与23%，倍量投与6%であった（Fig. 2）．3）「職員に対するHBs抗体の定期検査の有無」は，「あり」62%で，検査頻度は12カ月毎の採血が91%と最多であった（Fig. 3）．4）「HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性」について，「必要」63%で，このうち93%でHBs抗体価10 mIU/mL未満の時点で実施していた（Fig. 4）．

考　察

米国CDCガイドラインの発表を受けて，日本環境感染学会ガイドラインでも「ワクチン接種シリーズ後の抗体検査で免疫獲得と確認された場合，その後の抗体検査や追加のワクチン接種は必要ではない」という勧告を出した[5]．すなわち，1）透析患者，2）HIV感染者，3）造血幹細胞移植を受けた患者，4）化学療法や免疫抑制療法を受けた患者などのハイリスクグループ以外は追加のワクチン接種は必要ではないとするガイドラインである．確かに，集団免疫（医療機関として）の観点からは，医療従事者の肝炎発症と患者への2次感染を防ぐことが目標であり，コストベネフィットを考慮した米国のガイドラインは正しいと言えよう．

一方，個人免疫の観点からは肝炎も嫌だが，将来の肝がんも防ぎたい．すなわち，HBc抗体が陽性化する感染を防ぐことにより，肝炎，肝癌，さらにはHBV再活性化すべてを予防することが可能となる．実際に福祉の国であるイギリスのガイドラインでは，抗体低下時の追加接種を推奨しており，HBs抗体価10～100 mIU/mLの人でさえ，1回追加接種したのち5年ごとに1回追加接種を推奨している[6]．特に，1）医療従事者，2）透析患者，3）パートナーや家族内にHBVキャリアがいる場合は強く推奨される．興味深いことに，今回の日本肝臓学会評議員などを対象としたアンケート調査では，「HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性」について，「必要」が63%で最も多く，その施設の多くは職員に対するHBs抗体の定期検査を12カ月ごとに行い，HBs抗体価10 mIU/mL未満の時点でHBワクチンを追加接種していた．これらの結果を踏まえると，「追加のワクチン接種は必要ではない」とする日本環境感染学会ガイドラインについて再度議論する必要があるように思われる．これは“B型肝炎”を「肝臓病」として捉えている肝臓専門医と「感染症」として捉えている感染症専門医との間にある根本的な考え方の相違に起因するものかもしれない．

これまでに医療従事者を何百人も対象とした研究や男性同性愛者やエスキモーを対象とした研究が長期間実施されており，これらの研究の成果はCDCからの勧告を支持しているが，HBc抗体が検出された症例が存在するのも事実である[7)～9)]．HBc抗体はHBVワクチンでは獲得されない抗体であり，この存在はHBV自体が体内に入り込み，免疫が反応したという根拠になる．すなわち，HBワクチン接種でHBs抗体陽性となった場合，その後のHBVへの曝露により肝炎を発症するこ

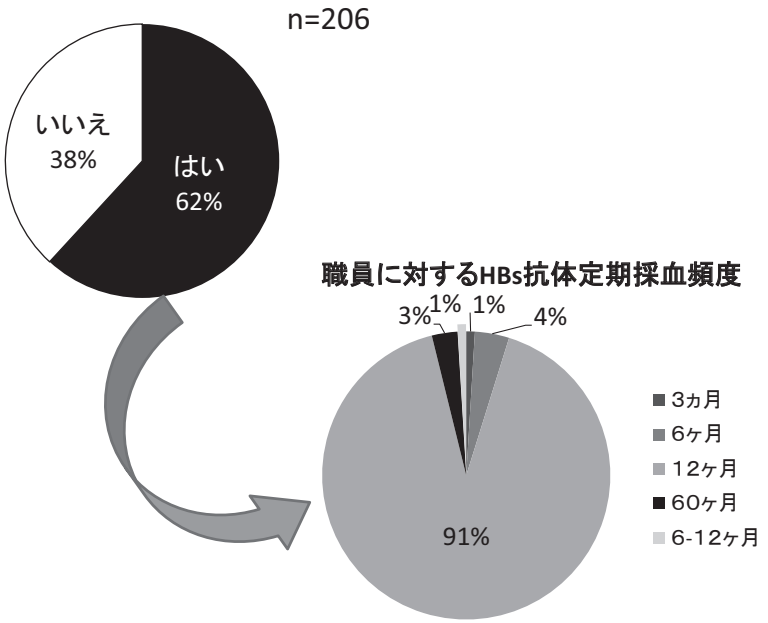

Fig. 3　職員に対する HBs 抗体の定期採血

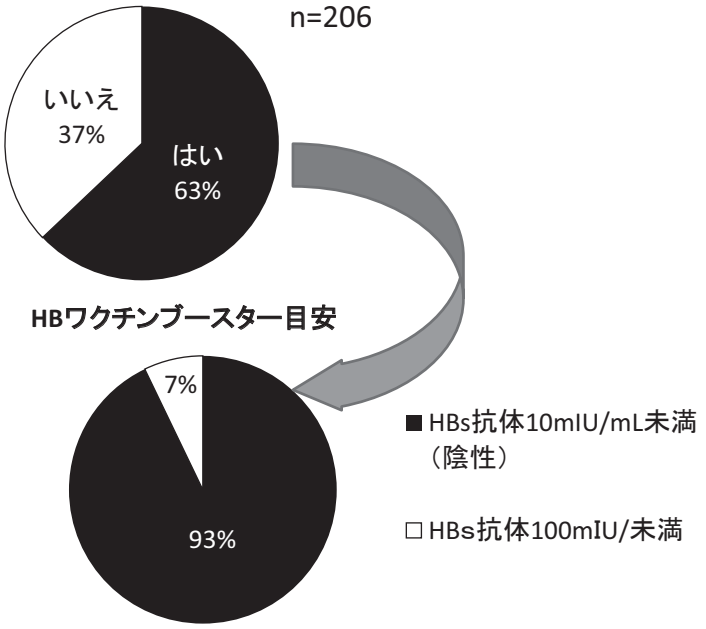

Fig. 4　医療従事者に対する HB ワクチンブースター

とはまれであるが，HBs 抗体価が低下した際には HBV への曝露後に HBV DNA が陽性となることがある[10]．このような状態はオカルト HBV 感染と称され，免疫抑制状態において HBV 再活性化を引き起こすことがある[11]．現在のところ，HB ワクチン接種後 HBs 抗体が陰転化した場合の HB ワクチン追加接種は推奨されていないが，HB ワクチン接種数年後に HBs 抗体価が低下し，急性肝炎（ALT 3,510 U/L）を発症した症例[12]や急性肝

炎発症（ALT 211 U/L）からキャリア化した症例[13]も報告されており，HBs抗体価10 mIU/mL未満に低下した場合にはHBワクチンを追加接種することも選択肢となりうる．特に，肝炎を発症しないまでも，HBc抗体が陽転化した時点で，肝臓内にはHBVはすでに侵入・感染していることになり，がん化学療法や免疫抑制剤使用時にHBV再活性化のリスクを背負うことになる．そのような予測可能な事態を肝臓専門医として容認してよいのか，今後も議論が必要と思われる．

結　語

日本肝臓学会評議員などを対象にアンケート調査を行った結果，HBワクチンに関する重要なエクスパートオピニオンが得られた．今後も，日本肝臓学会としての意見をまとめて広く情報発信する予定である．

謝辞：今回，HBワクチンの在り方を検討するための「日本肝臓学会HBワクチンワーキンググループ（企画広報委員会HBワクチン小委員会）」設立にご尽力頂きました小池和彦理事長ならびに企画広報委員会委員長の持田智先生に深く感謝申し上げます．なお，本アンケートにご協力いただきました日本肝臓学会役員及び評議員の先生方に深謝いたします．

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ORIGINAL ARTICLĒLIVER, PANCREAS, AND BILIARY TRACT

Epidemiologic features of 348 children with hepatitis C virus infection over a 30-year period: a nationwide survey in Japan

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Abstract

Background Although the epidemiology of hepatitis C virus (HCV) infection among children may be rapidly changing, few reports have characterized large nationwide cohorts of children with HCV infection. We, therefore, sought to clarify the epidemiology and natural history of HCV infection in Japanese children born over the last three decades.

Methods Sixty-five pediatric centers retrospectively and prospectively recruited consecutive, otherwise-healthy HCV-infected children born during 1986 to 2015.

Results Entry criteria were met by 348 children. Age at initial diagnosis of infection has decreased significantly in recent years. Cirrhosis and hepatocellular carcinoma were not identified. Prevalence of spontaneous clearance and of interferon treatment with/without ribavirin were 9 and 54%, respectively. Maternal transmission has increased significantly, representing over 99% of cases in the last decade. No transfusion-related cases have been seen after 1994. HCV genotype 2 has increased to become the most prevalent in Japanese children. Histopathology examination of liver specimens showed no or mild fibrosis in most children with chronic hepatitis C; none showed cirrhosis.

Conclusions This largest nationwide cohort study of Asian children with HCV infection spanned the last three decades. None of these Japanese children developed cirrhosis or hepatocellular carcinoma. Maternal transmission increased to account for 99% of cases during the last decade. Genotype 2 now is most prevalent in these children. Histopathologically, most children with chronic hepatitis C showed mild fibrosis or none.

Keywords Natural history · Maternal transmission · Genotype · Liver histopathology · Cirrhosis

Abbreviations

HCV	Hepatitis C virus
anti-HCV	Anti-HCV antibody
SVR	Sustained virologic response
IFN	Interferon
RBV	Ribavirin
CHC	Chronic hepatitis C
SD	Standard deviation
DAAs	Direct-acting antiviral agents

Introduction

Hepatitis C virus (HCV) infection is a major cause of liver disease. Recent estimates showed an increase in its worldwide prevalence over the last decade to 2.8%, amounting to

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over 185 million infections [1–3]. In Japan, estimated prevalence of HCV infection in adults has been 0.8 to 1.2% [4]. Prevalence is lower in children, estimated at 0.012% at ages 5–9 years, 0.010% at 10–14 years, and 0.022% at 15–19 years [5]. The low prevalence of HCV infection in children reflects disappearance of transmission by blood transfusions and other medical procedures, and also reduced mother-to-child (i.e., vertical or perinatal) transmission, even though this form of transmission currently is responsible for most new infections in developed countries [6–9]. Among HCV genotypes, genotype 1 is most prevalent worldwide (49.1%), followed by genotypes 3 (17.9%), 4 (16.8%), and 2 (11.0%). Genotypes 5 and 6 are responsible for the remaining infections, representing less than 5% [3]. In Japanese adults, relative prevalence of genotype 1 has declined while that of genotype 2 has increased; nonetheless, genotype 1 (65%) remains more prevalent than genotype 2 (34%) [4, 10]. Taken together, these data raise the question of possible rapid changes in the epidemiology of HCV infection among Japanese children, but few large nationwide cohort studies of children with HCV infection have been undertaken, particularly in the last decade [9, 11, 12]. To evaluate the extent of these changes, which could alter the future burden of HCV infection, we investigated epidemiologic features of a large nationwide cohort of children with HCV infection in Japan. Specifically, we aimed to clarify the epidemiology and natural history of HCV infection in Japanese children who were born over the last three decades.

Methods

Study design

This study was designed and conducted within the framework of the "Observatory for HCV Infection and Hepatitis C in Japanese Children," established in 2011 by the Hepatology Group of the Japanese Society for Pediatric Gastroenterology, Hepatology and Nutrition (JSPGHAN) with the aim of taking a census of children with HCV infection and investigating clinical aspects and outcomes of liver disease in this inadequately studied population. Sixty-five pediatric centers in Japan were involved in this survey. Over approximately 4 years, each of these centers retrospectively and prospectively collected all anti-HCV antibody (anti-HCV)-positive cases in children born from 1986 to 2015. Baseline and follow-up clinical information were obtained from patient records. Patient characteristics, clinical diagnosis at last visit, treatment, type of exposure, HCV genotype, and histopathologic features of liver biopsy specimens were determined. Features of the patients were evaluated in three groups defined by birth year: 1986–1995, 1996–2005, and 2006–2015. Some of these patients have been involved in previous studies [12–14]. The study protocol complied with the ethical guidelines of the Declaration of Helsinki of 1975 (2004 revision) and was approved by the ethics committee of Osaka General Medical Center and other participating centers.

Patients

Inclusion criteria were age between 0 and 16 years at initial diagnosis, birth between 1986 and 2015, HCV RNA positivity in at least one serum sample, follow-up for at least 1 year after the infection was diagnosed at the observatory center, and absence of coinfection with human immunodeficiency virus (HIV) or hepatitis B virus (HBV).

Clinical definitions were as follows. Spontaneous sustained clearance (in untreated HCV RNA-positive patients) signified disappearance of HCV RNA from at least two consecutive serum samples. Carriers were HCV RNA-positive patients with persistently normal serum alanine aminotransferase (ALT) concentrations. Chronic hepatitis was diagnosed in HCV RNA-positive patients with persistently increased ALT for more than 6 months or a liver biopsy specimen showing chronic hepatitis. Sustained virologic response (SVR) indicated HCV RNA negativity for 24 weeks following conclusion of interferon (IFN) treatment with/without ribavirin (RBV). Evidence of cirrhosis was diagnosed by liver biopsy or by clinical findings (jaundice, fatigue and/or edema), blood tests (hyperbilirubinemia, thrombocytopenia, hypoalbuminemia, and/or coagulopathy), and/or abdominal imaging including the liver using ultrasonography, computed tomography and/or magnetic resonance imaging (ascites, nodularity of the liver, and/or atrophy of the liver).

Type of HCV exposure

Putative types of HCV exposure were evaluated by concordant results of HCV genotype between mother and child and by ascertaining family history and past surgical and transfusion histories.

HCV RNA and genotype

HCV RNA was quantified in fresh or well-preserved stored sera by commercial quantitative assays such as real-time PCR (COBAS Ampliprep/COBAS TaqMan HCV test, Roche) in 90% of subjects, amplicor HCV monitor (COBAS Amplicor HCV Monitor test v 2.0, Roche) in 8% and branched DNA probe (Quantiplex HCV RNA 2.0, Bayer) in 2%. Genotype was assessed by genotyping assay using reverse transcription PCR of the core region with the genotype-specific primers in 82% of subjects and by serotyping assay in 18% according to the international classification [15, 16].

Histopathology

Histopathology of the liver was evaluated using initial liver biopsy specimens obtained from children with chronic hepatitis C (CHC) before they had received any IFN treatment with/without RBV. Liver biopsy specimens were assessed pathologically based on the New Inuyama Classification of chronic hepatitis [17], in which chronic hepatic disease is characterized according to degree of fibrosis (F) as follows: F0 (no fibrosis, equivalent to Ishak stage 0), F1 (fibrosis evident as portal expansion, equivalent to Ishak stage 1–2), F2 (bridging fibrosis, equivalent to Ishak stage 3), F3 (bridging fibrosis with lobular distortion, equivalent to Ishak stage 4), or F4 (cirrhosis, equivalent to Ishak stage 5–6) [17, 18]. Additionally, the classification assesses chronic hepatic disease activity (A) based on degree of lymphocytic infiltration and necrosis of hepatocytes as follows: A0 (no necro-inflammatory reaction), A1 (mild necro-inflammatory reaction), A2 (moderate necro-inflammatory reaction), and A3 (severe necro-inflammatory reaction) [17].

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) and categorical variables as frequencies and percentages. Chi squared, Fisher's exact, ANOVA, Tukey–Kramer, and Pearson correlation tests were used as appropriate. All statistical analysis was performed using GraphPad Prism version 6.05 software (GraphPad Software, San Diego, CA, USA). Tests were two-sided. *P* values below 0.05 were considered to indicate statistical significance.

Results

During this survey, participating centers enrolled 441 consecutive anti-HCV-positive children, among whom 348 children met entry criteria. Based on birth year, they were assigned to one of three groups: group 1, including 49 children born between 1986 and 1995; group 2, including 175 born between 1996 and 2005; or group 3, including 124 born between 2006 and 2015 (Fig. 1). Ninety-three children were excluded from this study for the reasons such as unknown RNA positivity, follow-up for less than 1 year, or presence of coinfection with HIV or HBV.

Patient features

Table 1 summarizes distribution of gender, age at initial diagnosis of infection, age at last clinical visit, clinical diagnosis at last visit, and treatment in the three groups.

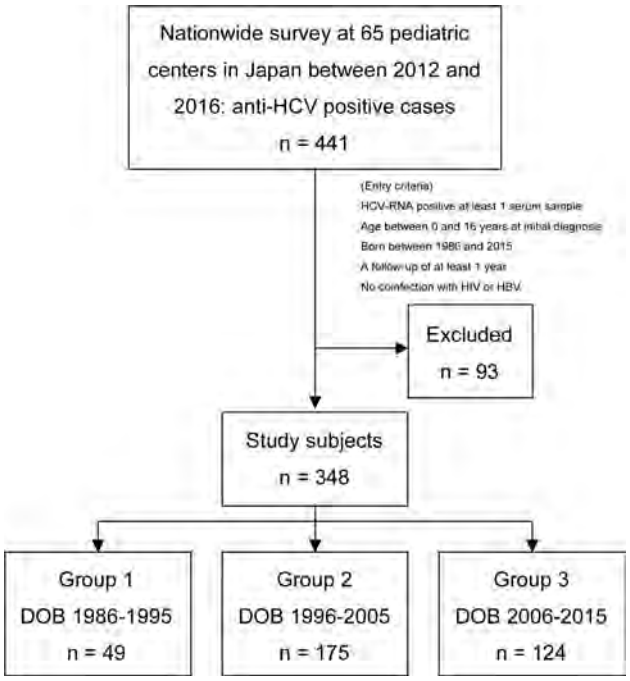

Fig. 1 Flow chart of this study. This chart summarizes entry criteria and distribution of patients into groups according to birth year. *HCV* hepatitis C virus, *anti-HCV* anti-HCV antibody, *n* number of patients, *HIV* human immunodeficiency virus, *HBV* hepatitis B virus, *DOB* date of birth

Girls accounted for 56% of patients. Age at initial diagnosis of infection had decreased significantly in recent years ($P < 0.0001$). As for clinical diagnosis at last visit, frequencies of spontaneous clearance, carrier state, chronic hepatitis, and SVR were 9, 34, 4, and 40%, respectively. Carriers had increased significantly in recent years ($P < 0.0001$), and SVR had decreased significantly ($P < 0.0001$). Cirrhosis and hepatocellular carcinoma were not identified. The overall fraction of patients who received IFN treatment with/without RBV in recent years was 54%, having decreased significantly ($P < 0.0001$).

Type of HCV exposure

Table 2 characterizes the 348 children based on putative type of exposure to HCV in the three groups. Maternal transmission, the most frequent source of infection in all groups, accounted for 90% of infections overall, with a significant increase in recent years ($P < 0.0001$), increasing to over 99% in the last decade. Transfusion was the second most frequent source of infection in the earliest decade, while no transfusion-related cases have been seen since 1994. Only 17 cases (5%) were ascribed to other putative sources of infection, horizontal transmission or unknown source.

Table 1 Demographic and clinical features of the 348 children enrolled in the study

	Total ($n = 348$)	Group 1 1986–1995 ($n = 49$)	Group 2 1996–2005 ($n = 175$)	Group 3 2006–2015 ($n = 124$)	P values[a]
Male, n (%)	154 (44)	21 (43)	79 (45)	54 (44)	0.9418
Age at diagnosis of infection, months[b,f]	37.7 ± 45.2	76.7 ± 59.6	43.0 ± 44.1	13.0 ± 16.0	<0.0001
Age at last visit, months[b,f]	130.7 ± 70.2	240.6 ± 49.6	148.9 ± 38.0	61.7 ± 28.8	<0.0001
Clinical diagnosis at last visit, n (%)					
Spontaneous clearance	30 (9)	1 (2)	13 (8)	16 (13)	0.0525
Carrier[c]	120 (34)	9 (19)	45 (26)	66 (53)	<0.0001
Chronic hepatitis	15 (4)	1 (2)	6 (3)	8 (6)	0.3134
Sustained virologic response[d]	139 (40)	33 (67)	88 (50)	18 (15)	<0.0001
During treatment	16 (5)	1 (2)	9 (5)	6 (5)	0.6488
Unknown	28 (8)	4 (8)	14 (8)	10 (8)	0.9993
Cirrhosis/HCC	0/0				
Treatment (IFN with/without RBV), n (%)[e]	188 (54)	37 (76)	118 (67)	33 (27)	<0.0001

n number of patients, *HCC* hepatocellular carcinoma, *IFN* interferon, *RBV* ribavirin

[a] Comparison among the 3 groups by Chi squared or ANOVA tests

[b] $P < 0.0001$, Group 1 vs. Group 2, Group 1 vs. Group 3, and Group 2 vs. Group 3 by Tukey–Kramer test

[c] $P < 0.0001$, Group 1 vs. Group 3 and Group 2 vs. Group 3 by Fisher's exact test

[d] $P = 0.0364$, Group 1 vs. Group 2; $P < 0.0001$, Group 1 vs. Group 3 and Group 2 vs. Group 3 by Fisher's exact test

[e] $P < 0.0001$, Group 1 vs. Group 3 and Group 2 vs. Group 3 by Fisher's exact test

[f] Mean ± standard deviation

Table 2 Putative types of exposure to HCV infection in 348 children

	Total ($n = 348$)	Group 1 1986–1995 ($n = 49$)	Group 2 1996–2005 ($n = 175$)	Group 3 2006–2015 ($n = 124$)	P values[a]
Maternal, n (%)[b]	314 (90)	30 (61)	161 (92)	123 (99)	<0.0001
Horizontal, n (%)	2 (1)	0	2 (1)	0	0.3700
Transfusion, n (%)[c]	17 (5)	17 (35)	0	0	<0.0001
Unknown, n (%)[d]	15 (4)	2 (4)	12 (7)	1 (1)	0.0398

n number of patients

[a] Comparison among the three groups by Chi squared test

[b] $P < 0.0001$, Group 1 vs. Group 2 and Group 1 vs. Group 3; $P = 0.0054$, Group 2 vs. Group 3 by Fisher's exact test

[c] $P < 0.0001$, Group 1 vs. Group 2 and Group 1 vs. Group 3 by Fisher's exact test

[d] $P = 0.0176$, Group 2 vs. Group 3 by Fisher's exact test

HCV genotype

Table 3 characterizes 298 of the children based on the HCV genotypes in the three groups. Overall relative prevalences of genotypes 1, 2, and 3 were 42, 57, and 1%, respectively. Genotype 1 has decreased significantly in recent years ($P = 0.0427$), while genotype 2 has increased ($P = 0.0775$).

Histopathology

Table 4 summarizes the demographic and clinical features of 147 children with CHC who underwent liver biopsy between 1995 and 2015, while Table 5 presents the histopathologic features of the liver according to the New Inuyama Classification [17]. Mean age at biopsy was 8.9 ± 4.0 years. The distribution of degree of necro-

Table 3 HCV genotype in 298 children

	Total ($n = 298$)	Group 1 1986–1995 ($n = 44$)	Group 2 1996–2005 ($n = 158$)	Group 3 2006–2015 ($n = 96$)	P values[a]
Genotype 1, n (%)[b]	126 (42)	25 (57)	68 (43)	33 (34)	0.0427
Genotype 2, n (%)	169 (57)	19 (43)	89 (56)	61 (64)	0.0775
Genotype 3, n (%)	3 (1)	0	1 (1)	2 (2)	0.4095

n number of patients

[a] Comparison among the three groups by Chi squared test

[b] $P = 0.0162$, Group 1 vs. Group 3 by Fisher's exact test

Table 4 Demographic and clinical features of 147 children with chronic hepatitis C who underwent liver biopsy between 1995 and 2015

Male, n (%)	70 (48)
Age at biopsy, years[a]	8.9 ± 4.0
Duration of infection, years[a] (maternal transmission, $n = 127$)	8.4 ± 3.6
Type of exposure, n (%)	
Maternal	127 (86)
Transfusion	10 (7)
Horizontal or unknown	10 (7)
HCV genotype ($n = 131$), n (%)	
Genotype 1	63 (48)
Genotype 2	66 (50)
Genotype 3	2 (2)

n number of patients

[a] Mean ± standard deviation

Table 5 Histopathologic features of liver biopsy specimens from 147 children with chronic hepatitis C

N (%)	A0 (5)	A1 (74)	A2 (20)	A3 (1)
F0 (33)	6	34	8	0
F1 (58)	2	70	12	1
F2 (9)	0	5	9	0

n number of patients, *A0* no necro-inflammatory reaction, *A1* mild necro-inflammatory reaction, *A2* moderate necro-inflammatory reaction, *A3* severe necro-inflammatory reaction, *F0* no fibrosis, *F1* fibrosis with portal expansion, *F2* bridging fibrosis

inflammatory activity (A0, A1, A2, and A3) was 5, 74, 20, and 1%, respectively. The distribution of degree of fibrosis (F0, F1, and F2) was 33, 58, and 9%, respectively. F3 and F4 were not seen. No significant correlation was found between degree of fibrosis and age at biopsy or duration of infection (Supplementary Figs. 1 and 2). Degree of fibrosis was not related to gender, type of exposure, or genotype (Supplementary Tables 1 to 3).

Discussion

Few reports describing large nationwide cohorts of children with HCV infection are available, although recent reports concerning adults indicate that the epidemiology of HCV infection is changing dramatically worldwide [1–3, 9, 11, 12]. We investigated the epidemiologic features of Japanese children with HCV infection to clarify natural history and trends over the last three decades. Previous large nationwide cohort studies of children with HCV infection describe epidemiologic features observed about two decades before 2006 [9, 11, 12]. Our investigation represents the largest nationwide cohort study of Asian children with HCV infection over a 30-year period, including children born during the most recent decade, 2006–2015. Additionally, we included a large pediatric-age survey of HCV histopathologic features, characterizing 147 children with CHC.

Since HCV was discovered in 1989 [19, 20], the Japanese Red Cross has screened blood donors for anti-HCV with a first-generation assay beginning in 1989, or, since 1992, a second-generation assay [21]. The present study shows that because of screening, transfusion transmission has decreased dramatically, and transfusion-related cases have disappeared after 1994. Three patients had putative transfusion transmission between 1992 and 1994, most likely because risk of fibrinogen-transmitted HCV infection was yet to be eliminated in Japan during that period [22]. At present maternal transmission accounts for 99% of cases, representing nearly the sole route for pediatric-age HCV infection. Comparing group 2 (born from 1996 to 2005) with group 3 (2006–2015), ages at time of diagnosis steadily decreased. We believe that this change reflects heightened awareness of maternal transmission of HCV among Japanese obstetricians and pediatricians; nearly all pregnant women in Japan now are screened for anti-HCV.

Girls were somewhat more numerous than boys among our subjects (56%) and spontaneous clearance occurred in 9% of patients, in essential agreement with previous reports [9, 11, 23]. IFN treatment with/without RBV was given to 54% of patients. Suzuki et al. reported that pegylated IFN monotherapy and pegylated IFN combined with RBV both produced encouraging results against HCV infection and were well tolerated and reasonably safe in Japanese children and adolescents with CHC, including some enrolled in this study [13]. Interestingly, our survey identified no patients with cirrhosis. Bortolotti et al. reported that 2% of untreated children with HCV infection progressed to decompensated cirrhosis before 16 years of age [9]. We believe that none of our subjects showed cirrhosis because of racial differences, because roughly half of them received IFN therapy with/without RBV, or because of both factors.

Relative prevalence of HCV genotypes is changing worldwide. We found genotype 1 to be decreasing, as did a previous report of children with HCV infection in Italy [11]. Genotype 2 was increasing in our Japanese survey, in contrast with increases in genotypes 3 and 4 in Italy [11]. Notably, genotype 2 has become most prevalent (57%) in our pediatric survey, although a recent report concerning adults stated relative prevalences of genotypes 1 and 2 in Japan in 2011 as 65 and 34%, respectively [4]. Toyoda et al. reported that genotype 1 remains most common in adults born before 1970, although genotype 2 has become most prevalent in adults born in or after 1970. Additionally, about half of these younger infected adults had a history of intravenous drug use or tattooing (though not of blood transfusion) [24]. These results suggest that in Japan genotype 2 may have spread to young adults by drug use or tattooing and then to children by maternal transmission. Up-to-date knowledge of genotype frequencies in Japanese children will be important in considering future treatment options against HCV infection.

Histopathology examination of liver specimens from most children with CHC showed fibrosis to be absent or mild, with inflammation predominating. No cirrhosis was found. Table 6 summarizes the largest studies of liver biopsy findings in children with CHC from Europe, the US, and Japan [14, 25, 26]. Kage et al. reported that the liver showed absent or mild fibrosis in most untreated Japanese children with CHC, as well as absence of cirrhosis. However, transmission was different in that study, with transfusion accounting for 85% of cases [14]. In the present study, even though 86% of our patients who underwent liver biopsy had maternal transmission, we observed similar histopathologic features in untreated Japanese children with CHC, including absence of fibrosis in 33% of patients and absence of cirrhosis in all. In contrast, Guido et al. reported that liver histopathology showed cirrhosis in 1% of untreated children with CHC in Italy and Spain [25], while Goodman et al. found the frequency in the US to be 2% [26]. Additionally, fibrosis was absent in smaller percentages of specimens in these studies than ours (28% [25] and 14% [26] vs. 33%). Thus, Japanese children with CHC might have less risk of fibrosis and cirrhosis than chronically infected children in some Western countries. Some reports of adults with CHC have associated patient age and duration of infection with progression of fibrosis [27, 28]. In children with CHC, the present study and Goodman et al. showed no significant correlations of degree of fibrosis with age at biopsy or duration of infection, although Guido et al. found degree of fibrosis to correlate with both patient age and duration of infection [26, 29]. Additionally, Mohan et al. reported that sequential biopsy specimens demonstrated progression of fibrosis in children with CHC, aged 8.6 ± 4.1 years at the first biopsy and 14.5 ± 4.0 years at the second [30]. Accordingly, severity of fibrosis might be more closely related to age or duration of infection in adolescence and young adulthood than in childhood.

New direct-acting antiviral agents (DAAs) now are being developed at a remarkable pace. Combining DAAs targeting different stages in the viral proliferation cycle has proven highly effective, permitting development of IFN-free and largely RBV-free regimens that might be better tolerated. Such oral regimens now have shown cure rates exceeding 90% in most adult populations [31–33]. We soon should be able to treat children with HCV infection using the new DAAs [34]. The results of our study, particularly, those concerning genotype trends and histopathologic features, should be useful to pediatric hepatologists in Japan and elsewhere in considering treatment of children with HCV infection using the new DAAs.

HCV/HIV coinfection is highly prevalent in Asia [35]. Omata et al. reported that maternal transmission of HCV is affected significantly by coinfection with HIV, and safety and efficacy of recently developed DAAs and those under development in reducing maternal transmission, particularly in the presence of HIV coinfection, require further investigation [36]. In the present study, maternal transmission accounted for 99% in the last decade. We therefore should undertake curative treatment using new DAAs in young women with HCV/HIV coinfection before pregnancy in order to prevent maternal transmission.

An important limitation of this study is the retrospective nature of data from most patients, particularly those who are older. The group born from 1986 to 1995 is smaller than groups born from 1996 to 2005 or from 2006 to 2015, probably because of loss of patient record accessibility at pediatric centers following transition to adult health care. Clinical diagnosis at last visit and prevalence of treatment clearly differ between subjects born from 1986 to 2005 and

Table 6 Liver histologic findings in large studies of children with chronic hepatitis C

Author	Year	Country	Patients	Age at biopsy years, mean ± SD	Type of exposure, %		Fibrosis, %			
					Maternal	Transfusion	None	Mild	Bridging	Cirrhosis
Kage et al. [14]	1997	Japan	109	8.8 ± 4.2	11	85	96[a]		4	0
Guido et al. [25]	1998	Italy/ Spain	80	9.1 ± 4.8	60	24	28	55	16	1
Goodman et al. [26]	2008	US	121	9.8 ± 3.7	78	7	14	80	4	2
Present study	2017	Japan	147	8.9 ± 4.0	86	7	33	58	9	0

Fibrosis staging as follows: none, F0 or Ishak 0; mild, F1 or Ishak 1–2; bridging, F2-3 or Ishak 3–4; cirrhosis, F4 or Ishak 5–6

SD standard deviation

[a] Total of none and mild

those born from 2006 to 2015 because of differing length of the follow-up period.

In conclusion, we clarified the epidemiologic features and natural history of Japanese children with HCV infection over the last three decades. To our knowledge, this is the largest nationwide cohort study from Asia. Age at initial diagnosis of infection has decreased significantly. Cirrhosis and hepatocellular carcinoma did not develop. The proportion of maternal transmission significantly increased in the last decade to 99%. No transfusion-related cases have been seen since 1994. Genotype 2 has become most prevalent among Japanese children. Histopathologic examination of the liver showed fibrosis to be absent or mild in most children with CHC.

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Authors' contributions TM, TT, and HT contributed to the concept and design of the study. All authors contributed to analysis and interpretation of the data. TM and HT contributed to writing the manuscript. Thus, all authors contributed to the manuscript.

Compliance with ethical standards

Conflict of interest We have no conflict of interest.

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Short Communication

Suppression of hepatitis B surface antigen production by combination therapy with nucleotide analogues and interferon in children with genotype C hepatitis B virus infection

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Aim: Sustained suppression of hepatitis B surface antigen (HBsAg) production after interferon (IFN) treatment has not been reported for children with genotype C chronic hepatitis B virus (HBV) infection, which is prevalent in Asia. Among children with hepatitis B envelope antigen-positive genotype C chronic HBV infection, we compared the efficacy of combination therapy with nucleotide analogues and IFN-α in 11 children with 12 historical cases treated with IFN monotherapy.

Methods: The combination of lamivudine and conventional IFN-α was introduced for the first three patients; the other eight patients were treated with entecavir and pegylated IFN.

Results: Demographic factors as well as baseline HBsAg titers and HBV-DNA levels were similar between the two groups. In the combination therapy group, viral loads were suppressed in 9/11 to below 4.0 log copies/mL both at the end of the therapy (EOT) and at 6 months after EOT. In contrast, in the IFN monotherapy group, suppression of viral loads was observed in 2/12 and 3/12 at EOT and at 6 months after EOT, respectively. In the combination therapy group, HBsAg titers dropped from 4.03 at pretreatment to 2.91 log IU/mL at 6 months after EOT with 4/11 showing a drop to below 1000 IU/mL (one patient achieved HBsAg clearance). In contrast, the amount of HBsAg did not change during the corresponding periods in the IFN monotherapy group.

Conclusions: Our preliminary results suggest that combination therapy might be effective in the suppression of HBsAg production as well as HBV-DNA production for children with genotype C chronic HBV infection.

Key words: genotype C, HBeAg seroconversion, HBsAg seroconversion, interferon, nucleotide analogue

INTRODUCTION

INTERFERON (IFN) IS a standard therapy of care for children with chronic hepatitis B virus (HBV) infection.[1] However, IFN monotherapy has not been satisfactory in promoting hepatitis B surface antigen (HBsAg) clearance in children or adults in Japan.[2] Moreover, sustained suppression of HBsAg production after IFN treatment was not reported for children with chronic hepatitis B, including genotype C chronic HBV infection, which is prevalent in Asia.

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In adult patients, HBsAg loss after tenofovir plus pegylated interferon-α (PEG-IFN) therapy was recently reported and suppression of HBsAg production by combination therapy was associated with HBV genotype A.[3] Our survey of published work failed to find any reports on the efficacy of this combination therapy in children with genotype C chronic HBV infection. In this study, we investigated the efficacy of combination therapy with nucleotide analogues and IFN-α in terms of suppression of HBsAg production as well as other biochemical and virological responses, including alanine aminotransaminase (ALT) normalization, hepatitis B envelope antigen (HBeAg) seroconversion, and suppression of HBV-DNA levels.

METHODS

FROM 2010 TO 2016, 39 patients with HBeAg-positive genotype C chronic HBV infection and their guardians

visited our center. Twenty-one of the 39 patients who had a sustained elevation in ALT for more than 6 months had the therapy explained to them. Eleven of the 21 agreed to enroll in the trial therapy (combination therapy group) whereas the other 10 patients had therapy withheld. The remaining 18 had never experienced an elevation in ALT levels and were regarded as asymptomatic carriers. An elevation in ALT levels was defined as a level >60 IU/L according to Jonas *et al.*[1]

As a comparison, registered cases that had received IFN monotherapy or PEG-IFN monotherapy were searched using the medical records of children with chronic HBV infection, which were collected in a nation-wide survey.[4] We identified 82 patients with IFN monotherapy and 14 patients with PEG-IFN monotherapy. Among them, 12 patients with IFN monotherapy and four patients with PEG-IFN monotherapy met the following inclusion criteria: pretreatment HBeAg positivity, availability of laboratory data including ALT, HBsAg, HBeAg, and HBV-DNA both at baseline and at 6 months after the end of therapy (EOT), and completion of the scheduled treatment regimen as described below. On evaluation of an efficacy of combination therapy, only cases with IFN monotherapy were compared because the number of eligible cases with PEG-IFN monotherapy was too small to compare with the combination therapy group.

The effect on HBsAg production as well as circulating levels of ALT, HBeAg, and HBV-DNA were assessed prior to therapy, at EOT, and every 6 months after EOT in the 11 children with genotype C chronic HBV infection. Liver biopsy specimens were evaluated for the activity of hepatitis and the degree of fibrosis according to the classification of Desmet *et al.*[5]

Treatment regimen

Combination therapy consisted of nucleotide analogues for the first 3 months using lamivudine 3 mg/kg/day plus natural IFN-α 0.1 MU/kg body weight three times a week for 6 months in the first three patients, or entecavir 0.01 mg/kg/day plus PEG-IFN 180 μg/m^2 body surface area weekly for 6 months in the remaining eight patients. The IFN monotherapy group received natural IFN-α 0.1 MU/kg body weight three times a week for 24 weeks. The PEG-IFN monotherapy group received 180 μg/m^2 body surface area weekly for 48 weeks.

Statistical analysis

Differences in mean values and the frequency of patients' characteristics between groups were compared using the Mann–Whitney *U*-test and the Fisher's exact test, respectively. All statistical analyses were based on two-sided hypotheses tested with a significance level of $P < 0.05$.

Ethical considerations

The study protocol complied with the ethical guidelines of the Declaration of Helsinki of 1975 (2004 revision) and was approved by the Ethics Committee of Osaka General Medical Center (Osaka, Japan).

RESULTS

Demographic data of children with HBeAg-positive genotype C chronic HBV infection

THE 11 CHILDREN who underwent the combination therapy from 2010 to 2016 consisted of seven boys and four girls with the average age of 9.2 years at treatment (Table 1). Transmission routes were mother to child in nine patients, father to child in one patient, and grandfather to child in one. Baseline factors including age at treatment, gender, transmission routes, and duration of observation were similar between the two groups. Baseline ALT values were greater in the combination therapy group than in the IFN monotherapy group, although it did not reach statistical significance. Both baseline HBsAg titers and HBV-DNA levels were in a similar range when comparing the two groups. A liver biopsy showed a mild activity of hepatitis (A1) for all patients expect one with a

Table 1 Comparison of demographic factors among children with genotype C hepatitis B virus (HBV) infection treated with interferon (IFN) monotherapy or combination therapy

	IFN monotherapy ($n = 12$)	Combination therapy ($n = 11$)	*P*-value
Age, years†	9.2 ± 4.2	9.2 ± 2.9	NS
Male sex, *n* (%)	4 (33)	7 (62)	0.22
MTCT, *n* (%)	8 (66)	9 (81)	NS
Observation, years†	4.0 ± 1.7	3.4 ± 2.1	0.45
Baseline ALT, IU/L†	155 ± 91	440 ± 375	0.06
Peak ALT, IU/L†	450 ± 605	664 ± 346	0.41
HBsAg, log IU/mL†	4.00 ± 0.30	4.23 ± 0.24	0.11
HBV-DNA, log copies/mL			
≥9	4	4	NS
8.0–8.9	4	5	
7.0–7.9	4	2	

†Mean ± standard deviation.
ALT, alanine aminotransaminase; IFN, interferon; MTCT, mother-to-child transmission; NS, not significant.

moderate degree of hepatitis (A2) (data not shown). A moderate degree of fibrosis (F2) was noted in all patients.

Natural course of children who had combination therapy withheld

Ten patients were followed for ALT, HBsAg, HBeAg, and HBV-DNA with no treatment for a median of 2.7 years. One of the 10 has had spontaneous seroconversion to HBeAb positive/HBeAg negative after 16 months of follow-up. In the remaining nine patients, HBeAg has remained positive.

Outcome of children with combination therapy or IFN monotherapy

In the combination therapy group, titers of HBeAg were rapidly decreased during the 6 months of therapy in all patients and suppressed in the negative range in eight of the 11 at EOT. Thereafter a loss of HBeAg occurred in two patients and remained positive in one patient at 6 months after EOT (Fig. 1). Hepatitis B envelope antigen seroconversion was significantly higher in the combination therapy group than in the untreated group (90.9% vs. 10.0%, $P \leq 0.001$). The seroconversion rate at 6 months after EOT was also greater in the combination therapy group than in the IFN monotherapy group ($P = 0.027$; Table 2a).

Viral loads were decreased in all patients of the combination therapy group during therapy and were suppressed in most of the patients to below 4.0 log copies/mL (LC/mL) both at EOT and at 6 months after EOT (Fig. 2a). In contrast, in the 12 patients of the IFN monotherapy group, the same degree of suppression of viral loads during the corresponding observation period was observed in only two and three patients at EOT and at 6 months after EOT, respectively (Fig. 2b). The decrease in viral loads at 6 months after EOT was more frequently seen in the combination therapy group than in the IFN monotherapy group ($P = 0.012$; Table 2a).

In the combination therapy group, HBsAg titers substantially dropped from 4.03 at pretreatment to 2.91 log IU/mL at 6 months after EOT: five of the 11 patients showed more than a 1.0-log drop in the HBsAg titers and in four of the five patients it decreased to <1000 IU/mL (Fig. 3a). Of note, one of the five patients achieved HBsAg clearance at 12 months after EOT (case 3). In contrast, the HBsAg levels did not change during the corresponding observation period in the IFN monotherapy group (Fig. 3b). The difference between the two

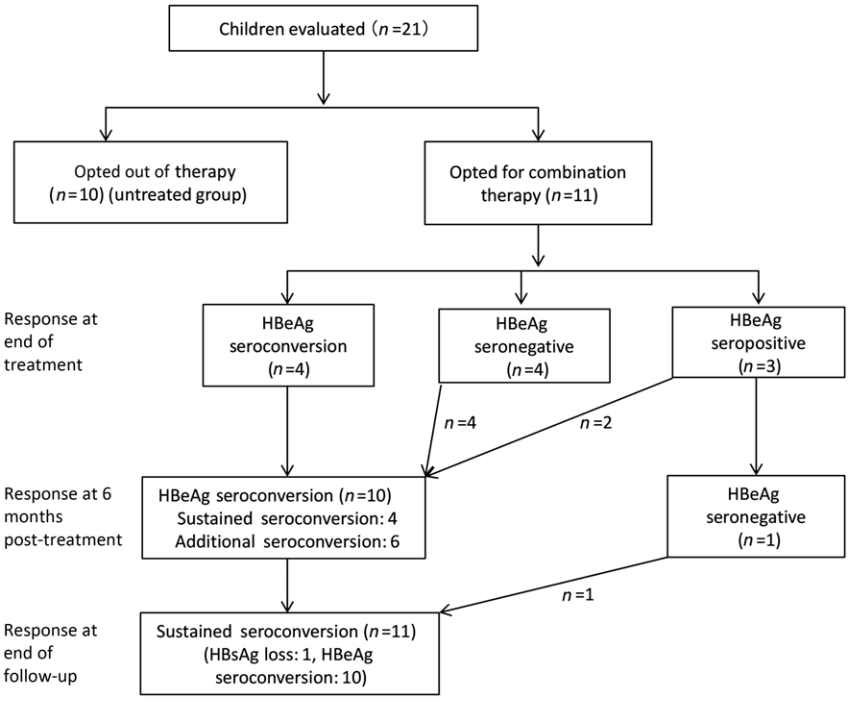

Figure 1 Flow diagram of the study of the efficacy of combination therapy with nucleotide analogues and interferon in children with genotype C hepatitis B virus infection, including summary of results. HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen.

Table 2a Comparison of efficacy between interferon (IFN) monotherapy and combination therapy groups among children with genotype C hepatitis B virus (HBV) infection

	Lamivudine plus interferon ($n = 3$)	Entecavir plus PEG-IFN ($n = 8$)	Combination therapy ($n = 11$)*	IFN monotherapy ($n = 12$)*	*P*-value*
ALT normalization	3/3	7/8	10/11	6/12	0.069
HBeAg/HBeAb seroconversion	3/3	7/8	10/11	5/12	0.027
HBV-DNA <4.0 log copy/mL	3/3	6/8	9/11	3/12	0.012
HBsAg 1.0-log drop	2/3	3/8	5/11	0/12	0.014
HBsAg <1000 IU/mL	1/3	3/8	4/11	0/12	0.037
HBsAg loss	1/3	0/8	1/11	0/12	NS

**P*-values are shown for these two groups.
ALT, alanine aminotransaminase; HBeAb, hepatitis B envelope antibody; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; NS, not significant; PEG-IFN, pegylated IFN.

Table 2b Comparison of side-effects between interferon (IFN) monotherapy and combination therapy among children with genotype C hepatitis B virus infection

	IFN monotherapy ($n = 12$)	Combination therapy ($n = 11$)	*P*-value
Leukopenia	2	1	NS
Anemia (Hb <10 g/dL)	0	0	NS
Thrombocytopenia (plt <100 000/μL)	1	1	NS
Elevated serum transaminase levels	2	1	NS
Hypothyroidism	0	0	NS
Lethargy	1	0	NS
Mental depression	0	0	NS
Hair loss	0	0	NS
Skin rash	0	0	NS

Hb, hemoglobin; NS, not significant; plt, platelets.

groups at 6 months after EOT was greater in the combination therapy group than in the IFN monotherapy group both for 1.0-log drop and for a drop below 1000 IU/mL ($P = 0.014$ and $P = 0.037$, respectively; Table 2a).

There were no differences between the first three patients treated with lamivudine plus interferon and the later eight patients with entecavir plus PEG-IFN in terms of seroconversion rate, suppression of viral loads, 1.0-log drop in HBsAg, or drop below 1000 IU/mL at 6 months after EOT (Table 2a).

Sustainability of the suppression of HBsAg production was partly shown by an 84-month follow-up in cases 2 and 3, both of which showed more than 1.0-log drop at 6 months after the end of the combination therapy (Fig. S1). Moreover, HBsAg titers decreased below 1000 IU/mL after 6 years in case 2. In the IFN monotherapy group, titers of HBsAg were available for most patients between 12 and 36 months after EOT and showed no change compared to those at 6 months after EOT (data not shown).

Outcome of children treated with PEG-IFN monotherapy

In the four patients who underwent PEG-IFN monotherapy, ALT normalization was reported in three, HBeAg seroconversion in two, and suppression of HBV-DNA in two at 6 months after EOT. The amount of HBsAg was repeatedly assessed in three of the four patients and no apparent decrement in HBsAg titers was observed in those three patients, either at EOT or 6 months after EOT.

Safety of combination therapy

A similar frequency of bone marrow suppression associated with IFN treatment was observed in the two groups; leukopenia in two and thrombocytopenia in one for the IFN monotherapy group, and one each for the combination therapy group (Table 2b). Transient elevation in serum transaminase levels was also infrequently seen in

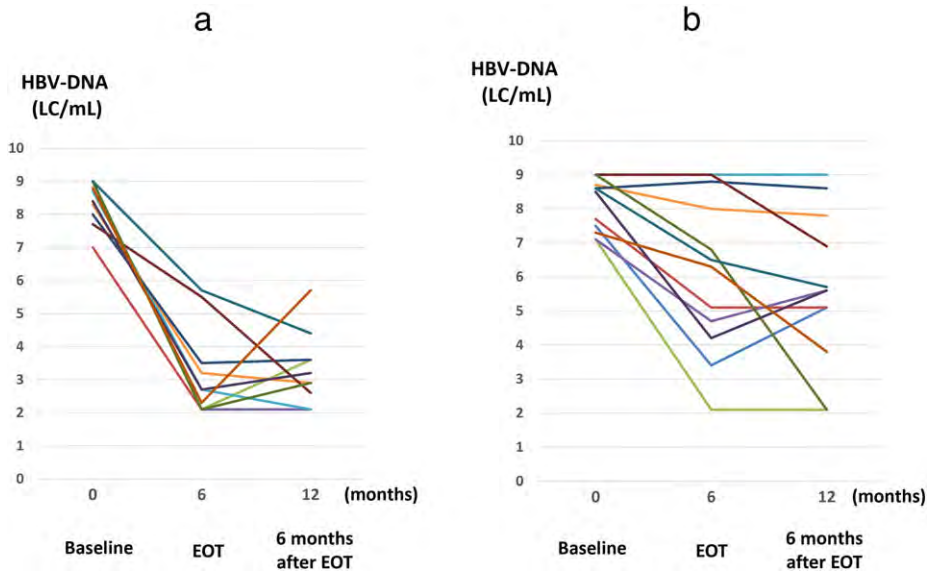

Figure 2 Hepatitis B virus (HBV)-DNA levels in two groups of children with genotype C HBV infection treated with combination therapy or interferon (IFN) monotherapy. Baseline values of each group are presented with corresponding estimations at end of treatment (EOT) and at 6 months after EOT for the combination therapy group (a) and the IFN monotherapy group (b). LC, log copies. [Color figure can be viewed at wileyonlinelibrary.com]

both groups. None of these side-effects was serious enough to warrant cessation of therapy.

DISCUSSION

IN THIS STUDY, all the 11 treated children showed a favorable response to combination therapy with IFN and nucleotide analogues. Suppression of HBeAg production occurred and serum HBV-DNA levels dropped to <4.0 LC/mL at 6 months after EOT in most patients. The mean value of HBsAg decreased from 4.03 log at baseline to 2.91 log IU/mL at 6 months among the 11 treated patients and HBsAg dropped below 1000 IU/mL in four patients. Furthermore, one of the four patients achieved

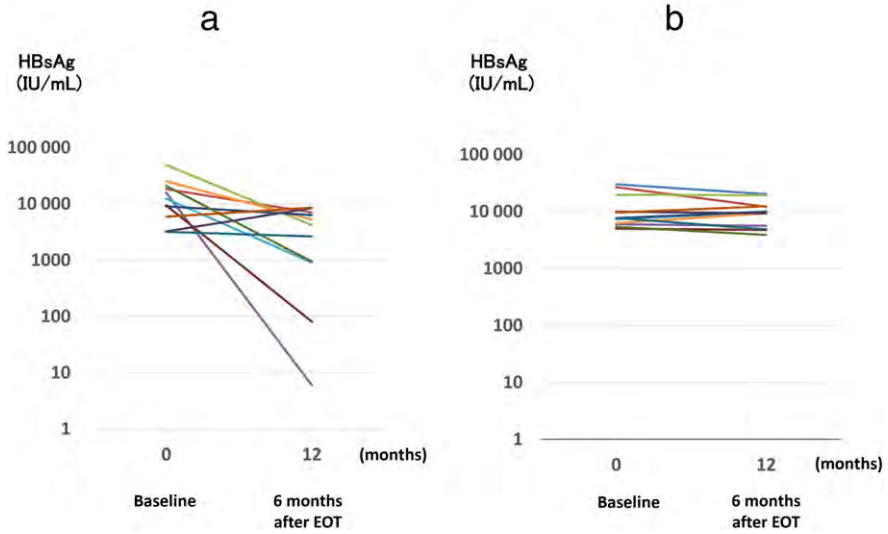

Figure 3 Hepatitis B surface antigen (HBsAg) titers (expressed as logarithms) in two groups of children with genotype C HBV infection treated with combination therapy or interferon (IFN) monotherapy. Baseline values of each group are presented with corresponding estimations at 6 months after end of treatment (EOT) for the combination therapy group (a) and the IFN monotherapy group (b). [Color figure can be viewed at wileyonlinelibrary.com]

HBsAg clearance 1 year after therapy and it was decreased below 1000 IU/mL in another patient after 6 years. The safety profile of the combination therapy group was similar to the IFN monotherapy group and no serious side-effects were observed in either group.

The first therapeutic trial in children using a similar regimen was reported by D'Antiga *et al.* in 2006.[6] They treated 23 immune-tolerant children and achieved HBeAg seroconversion in five (22%) and HBsAg loss in four (17%). All of the four patients who cleared HBsAg had genotype B HBV infection. Two of their 23 patients who had genotype C infection did not respond to the therapy. Similar combination therapy in 112 children with an ALT >1.5 times the upper limit of normal resulted in a higher response (55% vs. 27%) and more HBsAg loss (12.5% vs. 4.6%) when compared with 52 children who underwent nucleotide analogue lead-in combination therapy.[7] Twenty-eight children in an immune-tolerant phase were treated with combination therapy as reported by D'Antiga et al.[8] Eleven of the 28 become seronegative for HBeAg and five of the 11 had HBsAg clearance, but the genotype of the subjects was not examined in the latter two studies. Furthermore, these studies into the efficacy of combination therapy did not quantitatively assess the change in HBsAg production.

There have been no studies on the efficacy of combination therapy in children with genotype C chronic HBV infection. Therefore, it is unknown whether genotype C-infected children would respond to combination therapy with comparable efficacy as has been seen with genotype B in children.[6] A 20-year observation of the natural course of infection in children has shown that those with initial titers of HBsAg <1000 IU/mL were more likely to clear HBsAg than those with higher titers.[9] Accordingly, treatment-related suppression of HBsAg production <1000 IU/mL might lead to clearance of HBsAg in the near future. In this study, four of the 11 patients have achieved a suppression of HBsAg production <1000 IU/mL after the combination therapy. However, long-term observation is required to determine whether clearance of HBsAg might occur in the combination therapy group, as seen in children who showed low baseline levels of HBsAg and eventually cleared HBsAg.[9]

Our preliminary results suggest that combination therapy could be effective in suppression of HBsAg production as well as in suppression of both HBeAg and HBV-DNA production for children with chronic genotype C HBV infection. Prospective studies are needed to evaluate the efficacy of combination therapy and to clarify predictive factors of its efficacy in children with genotype C chronic HBV infection.

ACKNOWLEDGMENTS

THIS RESEARCH WAS supported by the Japan Agency for Medical Research and Development (grant no. 16fk0210310h0003).

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found online in the Supporting Information section at the end of the article.

Figure S1 Changes in hepatitis B surface antigen titers over 7 years for 11 children with genotype C hepatitis B virus infection treated with combination therapy.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Hepatitis B vaccine: Immunogenicity in an extremely low-birthweight infant

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Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan

Key words extremely low-birthweight infant, hepatitis B vaccine, hepatitis B virus, immunogenicity, mother-to-child infection.

From 2013, infants born to mothers carrying serum hepatitis B (HB) surface antigen (HBsAg) receive HB immunoglobulin at birth and HB vaccine at birth, and at 1 and 6 months of age in Japan (prevention protocol for mother-to-child HB virus infection).[1] Due to immature immune response to HB vaccine, the American Academy of Pediatrics and Japan Pediatric Society recommend that infants <2,000 g birthweight are given an additional HB vaccination at 2 months of age.[2,3] No previous case report, however, has described the trajectory of the immunogenic response for this prevention protocol, including an additional dose at 2 months of age, in extremely low-birthweight (ELBW) infants. The present case is reported with informed consent.

The present patient was born to a 29-year-old Chinese mother (gravida 0, para 0) with HBsAg. At 20 weeks of gestational age, serum HBsAg, HB envelope antigen, HB virus core-related antigen, and HB virus DNA were positive (67 878 IU/mL, 1,531.9 sample relative light units/cut-off, >7.0 log U/mL, and 9.7 log copies/mL, respectively). Both serum HB surface antibody (HBsAb) and HB envelope antibody were negative. The HB virus genotype was type C. A male newborn weighing 918 g was born at 25 weeks and 4 days of gestational age via cesarean section due to fetal distress.

He was admitted to the neonatal intensive care unit due to ELBW. Along with respiratory and circulatory treatment, i.v. immunoglobulin (IVIG; 500 mg/10 mL, Venoglobulin IH™, Japan Blood Products Organization, Tokyo, Japan) was administered soon after birth because of hypoimmune globulinemia (serum total IgG, 280 mg/dL). At 11 h after birth, a total of 200 U/mL HB immune globulin (Dried HB globulin Nichiyaku™; Nihon Pharmaceutical, Tokyo, Japan) was injected i.m. in the right and left femoral muscles (100 U/0.5 mL in each side), and HB vaccine (0.25 mL, Bimmugen™; Kaketsuken, Kumamoto, Japan) was injected s.c. in the left upper arm. No side-effects, such as redness, swelling, or induration were observed. HB vaccine was again administered at 1 and at 2 months of age. The infant was reared on breast milk and was discharged at 4 months of age. The fourth HB vaccine was injected at 6 months of age.

The HBsAb titer reached a peak at 1 month of age, and decreased to the lowest level at 4 months of age, but HBsAb was >10 mIU/mL (Fig. 1). Then, the HBsAb titer gradually increased, and after the fourth HB vaccine, it finally increased to >100 mIU/mL at 12 months of age. Serum HBsAg was negative at 12 months of age.

We herein report the HBsAb titer in an ELBW infant who received four doses of HB vaccine. In the present case, the prevention protocol for mother-to-child HB virus infection with an additional dose at 2 months of age (0, 1, 2, and 6 months of age) achieved sufficient seropositivity of HBsAb at 12 months of age. The infant had an HBsAb titer of 47 mIU/mL at the time of discharge, even with an additional vaccine at 2 months of age. Because ELBW infants are usually discharged from hospital at 3–4 months of age, and are

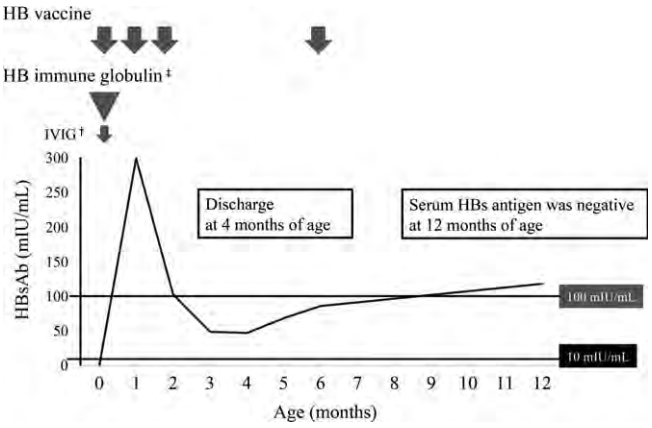

Fig. 1 Trajectory of serum hepatitis B surface antibody (HBsAb) titer. †Effect of i.v. immunoglobulin (IVIG) on HBsAb titer: the patient received 500 mg/10 mL Venoglobulin IH™ (Japan Blood Products Organization), which has an HBsAb titer of approximately 100 mIU/mL. Assuming that the circulating blood volume is 72 mL (80 mL/kg bodyweight) and the bioavailability of IVIG is 100%, IVIG treatment might have increased HBsAb titer by 14 mIU/mL. Given, however, that the half-life of Ig is 27 days,[4] the effect is limited. ‡Effect of HB immune globulin on HBsAb titer: the titer at 4 months of age (47 mIU/mL) can be explained only by the HB immune globulin at birth because the half-life of HB immune globulin is 23 days.[5]

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doi: 10.1111/ped.13547

then in close contact with their mother who are HB virus carriers, it is important for the ELBW infant to have a sufficient HBsAb titer at that time.

The seroprotection level is usually defined as HBsAb titer ≥10 mIU/mL.[6,7] Although all infants ≥2,000 g birthweight who received three doses of HB vaccine at 0, 1, and 6 months of age at the present hospital had sufficient HBsAb (median, 210 mIU/mL; range, 21–898 mIU/mL; n = 12), in a previous study, ELBW infants who received three doses of HB vaccinations at birth and at 1–3 and at 6–8 months of age had only a 52% seropositivity rate.[6] And in another study, 98.4% of preterm infants vaccinated using another four-dose HB vaccine protocol (0, 1, 2, and 12 months of age) had a protective level.[7] Four doses of HB vaccine may be needed to obtain a sufficient rate of seropositivity in ELBW infants as recommended by the Japan Pediatric Society.

Acknowledgments

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Disclosure

Outside the submitted work, I.M. has received grants from Japan Blood Product Organization, Daiichi Sankyo Co., Ltd., MSD Co., Ltd., AbbVie LLC, Taisho Toyama Pharmaceutical Co., Ltd., and Air Water Inc.; lecture fees from MSD Co., Ltd., Pfizer Japan, Inc., Novo Nordisk Pharma Ltd., Shionogi Co., Ltd., AbbVie LLC, Japan Vaccine Co., Ltd., Asahikasei Medical Co., Ltd., and Atom Medical Corp.; manuscript fees from Atom Medical Corp., Sanofi K.K., Asahikasei Medical Co., Ltd., and Japan Blood Product Organization,; and honoraria from Sanofi K.K. K.I. has received grants from Novartis Pharma K.K., Japan Blood Product Organization, Pfizer Japan, Inc., Kyowa Hakko Kirin Co., Ltd., AbbVie LLC, JCR Pharmaceuticals Co., Ltd., Daiichi Sankyo, Co., Ltd., Genzyme Japan K.K., Teijin Pharma Ltd., Miyarisan Pharmaceutical Co., Ltd., CSL Behring, Novo Nordisk Pharma Ltd., Air Water Inc., and Astellas Pharma Inc., Lecture fees from Pfizer Japan, Inc., Asahi Kasei Pharma Corp., Kowa Pharmaceutical Co., Ltd., MSD Co., Ltd., Alexion Pharmaceuticals, AstraZeneca K.K., Meiji Seika Pharma Co., Ltd., Novartis Pharma K.K., Zenyaku Kogyo Co., Ltd., Daiichi Sankyo, Co., Ltd., Springer Japan, Medical Review Co. Ltd., Chugai Pharmaceutical Co., Ltd., Boehringer Ingelheim, and Nikkei Radio Broadcasting Corporation, manuscript fees from Chugai Pharmaceutical Co., Ltd., and consulting fees from Zenyaku Kogyo Co., Ltd., Astellas Pharma Inc., Ono Pharmaceutical Co., Ltd. and Takeda Pharmaceutical Co., Ltd. The other authors declare no conflict of interest.

Author contributions

K.Y. and I.M. drafted the initial manuscript. K.Y. and S.I. collected the clinical data. K.Y., I.M. and K.F. interpreted the data. K.I. revised the article critically for important intellectual content. All authors contributed to the intellectual content of this manuscript and approved the final manuscript as submitted.

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厚生労働行政推進調査事業費（肝炎等克服緊急対策研究事業）

肝炎ウイルスの新たな感染防止
－残された課題・今後の対策－

平成 30 年度 総括・分担研究報告書

発行：平成 31(2019) 年 3 月

研究代表者 四柳　宏

東京大学医科学研究所先端医療研究センター 感染症分野

東京大学医科学研究所倫理審査委員会
審査結果通知書

平成３０年１２月２７日

申請者
感染症分野
四柳　宏　教授　殿

東京大学医科学研究所長
村上　善則

審査番号：　３０－６１
承認番号：　３０－６１－Ｂ１２２７
研究課題：　医療従事者へのB型肝炎ワクチン接種状況に関するアンケート調査
申請日：　平成３０年１２月２７日
審査委員会名：　倫理審査委員会第二委員会

上記研究計画について、平成３０年１２月２０日開催の本委員会における指摘事項の修正を確認し、下記のとおり決定しましたので、ここに通知します。

記

判定	
[承認]	~~変更の勧告~~
~~条件付き承認~~	~~否承認~~
□修正を要する	~~非該当~~
□修正不要	
理由・コメント	

整理番号	CRB-18-03-002
区分	□特定臨床研究■非特定臨床研究
	□医薬品□医療機器□再生医療等

2018年11月7日

臨床研究実施許可通知書

小児科・新生児科
髙野　智子　　様

2018年11月7日付け審査結果通知書にて承認された臨床研究について、実施を許可致します。

記

臨床研究課題名	保育の場における肝炎ウイルス感染予防の理解及び実践を図るための保育施設勤務者に対するアンケート調査

以上

大阪急性期・総合医療センター
総長

別記様式４

臨床研究倫理審査結果通知書

平成３０年１２月２８日

申請者（実施責任者）
岩淵　敦　殿

筑波大学附属病院長　　原　晃

平成３０年９月１３日付けで倫理審査申請のありました臨床研究の実施について、審査の結果、下記のとおり判定しましたので通知します。

記

１　臨床研究題目（H30-220）
「Ｂ型肝炎ワクチン定期接種化後の本邦小児におけるＢ型肝炎ウイルス感染およびワクチン接種の実態調査」

２　判　定
■　承　認
□　条件付承認
□　変更の勧告
□　不承認
□　非該当

３　理由等（判定が承認以外の場合）

研究期間　2018 年 12 月 28 日～2022 年 3 月 31 日
（ただし、臨床研究保険に加入する場合の研究開始日は、臨床研究保険補償開始日とする。）

整理番号

RK-190130-1

書式６　Ver. 1610

西暦２０１９年２月１３日

臨床研究　審査結果通知書

日本大学医学部附属板橋病院　病院長殿

日本大学医学部附属板橋病院
臨床研究倫理審査委員会
東京都板橋区大谷口上町３０番１号
委員長　武井　正美　　印

審査依頼のあった件について審査結果を下記のとおり報告いたします。

記

研究課題名	Ｂ型肝炎ワクチン定期接種化後の本邦小児におけるＢ型肝炎ウイルス感染及びワクチン接種の実態調査
審査事項 （審査資料）	■研究の実施の適否 ■臨床研究　申請書（西暦 2019年1月11日付） □臨床研究実施医療機関の概要書（西暦　年　月　日作成） □研究の継続の適否 □臨床研究　実施状況報告書（西暦　年　月　日付） □臨床研究　変更申請書（西暦　年　月　日付） □臨床研究における重篤な有害事象に関する報告書 （西暦　年　月　日付） □その他（　　）
研究期間	承認日　～　2022年3月31日
審査区分	■委員会審査（審　査　日：　2019年　2月 12日） □迅速審査　（審査終了日：　年　月　日）
審査結果	□承認　■条件付承認　□却下　□既承認事項の取り消し　□保留
指摘事項および理由・条件等	別紙(1902-07)のとおり
備考	別紙＜注意事項＞のとおり

西暦２０１９年 3 月 / 日

申請者(研究責任者)

小児・新生児病科

新生児病科外来医長　岡橋　彩　殿

申請のあった研究に関する審査事項について上記のとおり決定しましたので通知いたします。

日本大学医学部附属板橋病院　病院長　徳橋　泰明

2019 年 3 月 / 日 条件が満たされたことを確認しました。

日本大学医学部附属板橋病院　病院長

神小医第62号
平成31年3月25日

神戸大学大学院医学研究科内科系講座
小児科学分野こども急性疾患学部門
野津寛大　様

神戸こども初期急病セ
センター長　石田

神戸こども初期急病センター倫理委員会審査結果について(通知)

平成31年1月21日付けで倫理審査申請のありました「B型肝炎ワクチン定期接種化後の本邦小児におけるB型肝炎ウイルス感染およびワクチン接種の実態調査」について、倫理委員会委員長より、承認する旨の答申がありましたので通知いたします。

記

1. 答申日　平成31年3月25日

2. 参考資料　・答申書(写)

3. その他　当該研究に係る研究計画と経過、更に結果(成果)について継続的にセンターに報告し、寄附講座ホームページに掲載する等、広報に留意ください。

以上

様式２

国立感染症研究所ヒトを対象とする医学研究倫理審査結果通知書

平成３０年９月２５日

相崎　英樹　殿

国立感染症研究所長

受付番号：９２７

研究課題名：HIV感染同性愛者における急性Ａ型、Ｃ型肝炎の解析

研究者名：相崎　英樹・井戸田　一朗・三田　英治・遠藤　知之・四柳　宏・鈴木　亮介・清原　知子・杉山　隆一・村松　正道

研究期間：２０１８年承認日～２０２２年３月末日

上記課題名の研究計画・公表予定は、国立感染症研究所ヒトを対象とする医学研究倫理審査委員会において審議され、下記のとおり判定したので通知します。

記

判定	非該当	承認	条件付承認
	変更の勧告	不承認	
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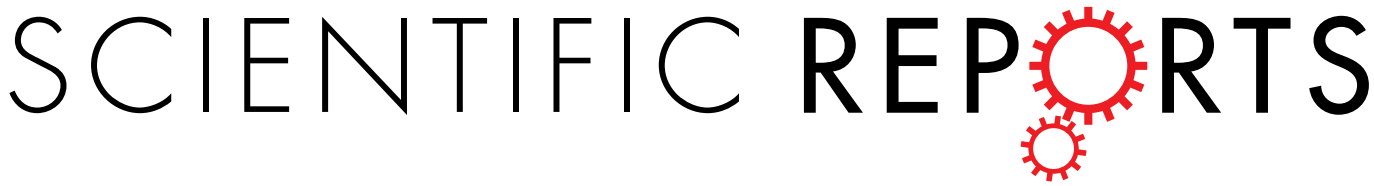

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Genome-wide association study identified new susceptible genetic variants in HLA class I region for hepatitis B virus-related hepatocellular carcinoma

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We have performed a genome-wide association study (GWAS) including 473 Japanese HBV (hepatitis B virus)-positive HCC (hepatocellular carcinoma) patients and 516 HBV carriers including chronic hepatitis and asymptomatic carrier individuals to identify new host genetic factors associated with HBV-derived HCC in Japanese and other East Asian populations. We identified 65 SNPs with P values $< 10^{-4}$ located within the HLA class I region and three SNPs were genotyped in three independent population-based replication sets. Meta-analysis confirmed the association of the three SNPs (rs2523961: OR = 1.73, $P = 7.50 \times 10^{-12}$; rs1110446: OR = 1.79, $P = 1.66 \times 10^{-13}$; and rs3094137: OR = 1.73, $P = 7.09 \times 10^{-9}$). We then performed two-field HLA genotype imputation for six HLA loci using genotyping data to

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investigate the association between HLA alleles and HCC. HLA allele association testing revealed that *HLA-A*33:03* (OR = 1.97, P = 4.58 × 10^{-4}) was significantly associated with disease progression to HCC. Conditioning analysis of each of the three SNPs on the HLA class I region abolished the association of *HLA-A*33:03* with disease progression to HCC. However, conditioning the HLA allele could not eliminate the association of the three SNPs, suggesting that additional genetic factors may exist in the HLA class I region.

Hepatitis B (HB) is a potentially life-threatening liver infection caused by hepatitis B virus (HBV), and approximately 248 million people worldwide are estimated to be chronically infected with HBV[1]. The clinical course of HBV infection is variable, including acute self-limiting infection, fulminant hepatic failure, inactive carrier state, and chronic hepatitis with progression to liver cirrhosis and hepatocellular carcinoma (HCC). Although some HBV carriers spontaneously eliminate the virus, every year 2–10% of individuals with chronic HB (CHB) develop liver cirrhosis, and a subset of these individuals suffer from liver failure or HCC[2]. Around 600,000 new HCC cases are diagnosed annually worldwide, and it is relatively common in Asia-Pacific countries and sub-Saharan Africa. More than 70% of HCC patients are diagnosed in Asia[3]. In contrast, HCC is relatively uncommon in the USA, Australia, and European countries[3,4]. The majority of HCC cases develop in patients with cirrhosis, which is most often attributable to chronic HBV infection followed by chronic hepatitis C virus infection in the Asia-Pacific region[5].

Human leucocyte antigen (HLA) proteins present self and non-self peptides to T cell receptors (TCRs) to maintain self-tolerance and adapted immunity. The HLA region resides on the short arm of chromosome 6, designated as 6p21.3. It is about 3.6 Mb in length and more than 200 functional and nonfunctional genes[6,7] are located in the region. The whole HLA region is divided into three subgroups, which are designated as class I, II, and III. The HLA class I region contains 19 HLA class I genes including 3 classical (*HLA-A*, *-B*, and *-C*), 3 non-classical (*HLA-E*, *-F*, and *-G*), and 12 non-coding genes or pseudogenes. The HLA class II region contains classical class II alpha- and beta-chain genes of *HLA-DR*, *-DQ*, and *-DP*. All HLA class I and class II molecules can present peptides to T cells, but each protein binds a different range of peptides. The presence of several different genes of each HLA class means that any one individual is equipped to present a much broader range of peptides than if only one HLA molecule of each class were expressed at the cell surface. A total of 17,695 *HLA* alleles (12,893 in class I and 4,802 in class II) were released by The IPD-IMGT/HLA database release 3.31.0 in January 2018 (https://www.ebi.ac.uk/ipd/imgt/hla/). Of the 12,893 class I alleles, 4,181, 4,950, and 3,685 alleles were registered in *HLA-A*, *-B*, and *-C* genes, respectively. Of 4,802 class II alleles, 2,146, 1,178, and 965 alleles were registered in *HLA-DRB1*, *-DQB1*, and *-DPB1* genes, respectively.

Recent genome-wide association studies (GWAS) of chronic HBV carriers with or without HCC in Chinese populations reported that one SNP (rs17401966) in *KIF1B*, two SNPs (rs9272105 and rs455804) in *HLA-DQA1/DRB1* and *GRIK1*, and two SNPs (rs7574865 and rs9275319) in *STAT4* and *HLA-DQ* were associated with disease progression to HCC[8–10]. A number of candidate genes have been investigated by genetic association studies to evaluate their roles in susceptibility to HCC. The findings from these studies, however, are inconclusive due to insufficient evidence and a lack of independent validation. All three papers referred to in this manuscript performed GWAS and replication studies using only Chinese population samples. For example, the study by Zhang *et al.*[10] used 2,310 cases and 1,789 controls of Chinese ancestry and identified one intronic SNP in *KIF1B* associated with HBV-related HCC. This result, however, was not replicated in several other populations[11,12]). These findings suggest that GWAS and subsequent replication studies should be conducted in populations other than Chinese.

In this study, we performed GWAS using Japanese CHB patients with and without HCC and a replication study using East Asian populations including Japanese, Hong Kong Chinese, and Thai.

Results

GWAS and replication study of HBV-related HCC. We conducted a GWAS using samples from 473 Japanese HBV-positive HCC patients and 516 HBV carriers including CHB and asymptomatic carrier (ASC) individuals by analyzing 447,830 autosomal SNPs. Figure 1 shows a genome-wide view of the SNP association data based on allele frequencies. There were 110 SNPs with P values < 10^{-4} in the GWAS (Supplementary Materials, Table S1). Of the 110 SNPs, 65 and 4 SNPs were located on the HLA class I and II regions, respectively. These results suggested that HBV-related HCC could be associated with SNPs located in the HLA region, although associations did not reach the genome-wide significance level. Outside the HLA region, there were 41 SNPs with P values < 10^{-4} and 4 SNPs showed P values < 10^{-5}.

In order to validate these suggestive associations, we selected seven SNPs based on the following criteria: P values < 10^{-4} in the HLA region and < 10^{-5} outside the HLA region and only SNPs with the lowest P value or highest OR were selected when multiple SNPs showed strong LD. Three independent sets of HBV-related HCC cases, CHB and ASC controls (replication-1: Japanese 153 cases and 614 controls; replication-2: Hong Kong Chinese 94 cases and 187 controls; and replication-3: Thai 185 cases and 198 controls), and the original GWAS set of 989 Japanese samples (473 cases and 516 controls) were genotyped and used in a subsequent replication analysis. Of the seven SNPs, four (rs2523961, rs1110446, and rs3094137 located on HLA class I region, and rs2295119 located on HLA class II region) were validated, and consistent associations were observed between the original GWAS set and replication sets (Table 1). For these four SNPs, no heterogeneity of association was observed between the original GWAS samples and the replication samples. Two SNPs in the HLA region (rs2523961 and rs1110446) showed a genome-wide significant association (rs2523961: OR = 1.91, P = 6.42 × 10^{-10}; and rs1110446: OR = 1.93, P = 2.52 × 10^{-10}) using the combined Japanese samples (GWAS and replication-1) (Table 1). Moreover, the meta-analysis with the combined Japanese samples and two independent sample sets (Hong Kong Chinese and

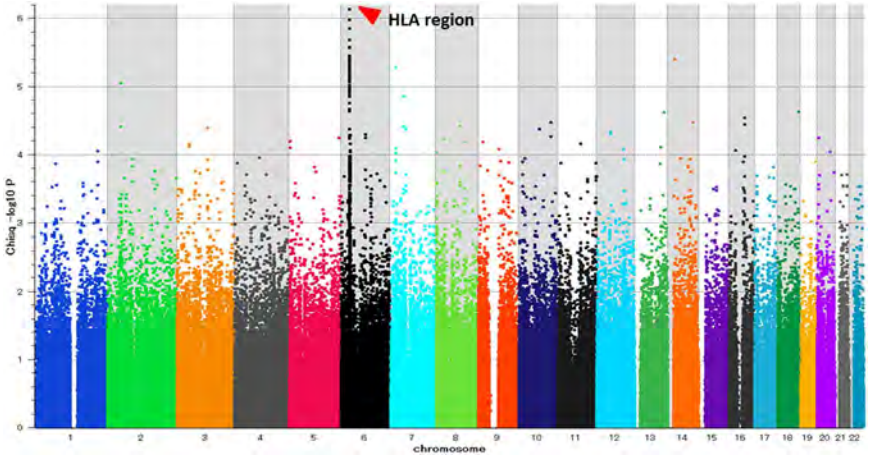

Figure 1. GWAS result. GWAS included 989 samples [473 Japanese HCC cases and 516 Japanese HBV carrier (CH and ASC) controls]. P-values were calculated using the chi-square test for allele frequencies among 447,830 SNPs.

Thai) confirmed associations for the two SNPs (rs2523961: $P = 5.81 \times 10^{-11}$; and rs1110446: $P = 9.09 \times 10^{-13}$), while the remaining two SNPs showed a marginal association (rs3094137: OR = 1.76, $P = 3.91 \times 10^{-7}$; and rs2295119: OR = 0.63, $P = 5.51 \times 10^{-7}$).

Association test for imputed HLA alleles. The two SNPs showing genome-wide significant associations were located on HLA class I region, and the marginally associated SNP was located on HLA class I and II region. To investigate the association of *HLA* alleles, we performed two-field HLA genotype imputation for six HLA loci (*HLA-A, -B, -C, -DRB1, -DQB1*, and *-DPB1*) using 989 genome-wide genotyping data used for the GWAS. Imputed *HLA* alleles were filtered (Call Threshold < 0.5) before performing association analysis for each HLA locus. The results of association tests in *HLA-A, -B, -C, -DRB1, -DQB1*, and *-DPB1* alleles are shown in Table 2 and Supplementary Materials, Table S2. To avoid false-positive results due to multiple testing for 77 HLA alleles, significance levels were set at 0.000649 (=0.05/77). A protective effect of *HLA-DPB1*02:01* (OR = 0.59, $P = 5.23 \times 10^{-6}$) was observed as previously reported[13]. We also detected that *HLA-A*33:03* was significantly associated with disease progression to HCC (OR = 1.97, $P = 4.58 \times 10^{-4}$) (Table 2).

Using GTEx-generated eQTL data[14], we checked for correlations between the three SNPs and *HLA-A* gene expression levels. The SNP rs2523961 was correlated with *HLA-A* gene expression in various tissues (muscle: $P = 6.1 \times 10^{-20}$; heart: $P = 2.3 \times 10^{-15}$, 2.1×10^{-11}; esophagus: $P = 2.8 \times 10^{-12}$, 1.8×10^{-6}; artery: $P = 4.7 \times 10^{-12}$, 3.9×10^{-11}; thyroid: $P = 1.4 \times 10^{-11}$; pancreas: $P = 3.3 \times 10^{-9}$; brain: $P = 1.9 \times 10^{-8}$, 2.2×10^{-7}; nerve: $P = 3.2 \times 10^{-8}$; testis: $P = 5.5 \times 10^{-7}$; lung: $P = 1.7 \times 10^{-5}$). The SNP rs1110446 was also associated with *HLA-A* gene expression in muscle ($P = 5.5 \times 10^{-15}$), skin ($P = 6.2 \times 10^{-11}$, 4.4×10^{-9}), artery ($P = 8.7 \times 10^{-6}$, 1.1×10^{-4}), esophagus ($P = 2.5 \times 10^{-5}$), and whole blood ($P = 5.1 \times 10^{-5}$). These results suggest that these SNPs affected *HLA-A* gene expression.

Conditioning each of the three SNPs on the HLA class I region (Supplementary Material, Fig. S1a–c) abolished the association of *HLA-A*33:03* ($P > 0.05$), but conditioning of *A*33:03* could not eliminate the association of the three SNPs (rs2523961: OR = 1.69, $P = 7.06 \times 10^{-4}$; rs1110446: OR = 1.65, $P = 9.33 \times 10^{-4}$; and rs3094137: OR = 1.54, $P = 5.68 \times 10^{-3}$) (Fig. 2). These conditional analyses suggest that additional genetic factors other than *HLA-A* allele exist in the HLA class I region. In contrast to the class I region, conditional analysis controlling for the SNP rs2295119 using *DPB1*02:01* allele suggests that *DPB1* allele could abolish the association of rs2295119 on the HLA class II region ($P > 0.05$) (Supplementary Material, Fig. S1e).

Discussion

In the current GWAS, we found a marginal association between an SNP (rs2295119) located in the *HLA-DPB* region and HBV-related HCC. Moreover, the association analysis of *HLA-DPB1* alleles and the conditional analysis with *HLA-DPB1*02:01* suggested that *DPB1*02:01* was the major protective allele in the HLA class II region. Recent GWAS also showed that SNPs located in the HLA class II region (*HLA-DQA1/DRB1*[9] and *HLA-DQ*[8]) were associated with HBV-related HCC in the Chinese population. We focused on the p-values of the HLA class II region (*HLA-DQ* and *-DR*) and six other gene regions (*KIF1B, UBE4B, PGD*, 8p12, *GRIK1* and *STAT4*) reported in previous studies and revealed the SNPs of four regions (*HLA-DQ* and *-DR*, 8p12, and *STAT4*) had p-values of less than 0.00625 (0.05/8). There were 52, 10 and 1 SNP with $P < 0.00625$ located on *HLA-DQ/DR*, 8p12, and *STAT4*, respectively, and the lowest p-value of each region was 0.00102 (rs9271894 on *HLA-DQA1*, OR = 1.46), 0.00278 (rs8084 on *HLA-DRA*, OR = 1.32), 0.00049 (rs13250548 on 8p12, OR = 0.68), and 0.0019 (rs6752770 on *STAT4*, OR = 1.44).

We also identified significant associations in the HLA class I region, especially around the *HLA-A* locus. The association test of imputed HLA alleles and conditional analyses with *HLA-A*33:03* suggested that *HLA-A*33:03* is the susceptibility allele for HCC. We performed additional conditional analyses controlling for the SNP on chromosome 6 using *A*33:03* and *DPB1*02:01* alleles. This indicated that *HLA-A* and *DPB1* alleles could

Marker	Allele (1/2)	stage	population	cases				controls				P value[b]	OR (95% CI)
				11	12	22	MAF	11	12	22	MAF		
rs2523961	A/G	GWAS	Japanese	12	174	287	0.209	11	111	394	0.129	2.57E-07	2.02 (1.54–2.66)
(class I)		Combined	Japanese	19	219	388	0.205	23	238	867	0.126	6.42E-10	1.91 (1.56–2.37)
		Replication2	Hong Kong Chinese	1	25	68	0.144	2	34	151	0.102	0.118	1.55 (0.90–2.66)
		Replication3	Thai	13	54	108	0.229	6	49	142	0.155	0.059	1.49 (0.98–2.28)
		Meta-analysis[a]										5.81E-11	
rs1110446	T/C	GWAS	Japanese	14	177	282	0.217	11	114	391	0.132	4.44E-08	2.10 (1.60–2.75)
(class I)		Combined	Japanese	21	222	383	0.211	24	245	861	0.130	2.52E-10	1.93 (1.57–2.37)
		Replication2	Hong Kong Chinese	2	22	70	0.138	1	35	151	0.099	0.138	1.52 (0.90–2.62)
		Replication3	Thai	14	66	100	0.261	5	51	142	0.154	0.002	1.93 (1.27–2.92)
		Meta-analysis[a]										9.09E-13	
rs3094137	A/G	GWAS	Japanese	9	150	314	0.178	10	97	409	0.113	9.65E-05	1.74 (1.31–2.31)
(class I)		Combined	Japanese	13	191	421	0.174	19	203	906	0.107	3.91E-07	1.76 (1.41–2.19)
		Replication2	Hong Kong Chinese	0	8	86	0.043	0	9	178	0.024	0.201	1.93 (0.71–5.21)
		Replication3	Thai	0	19	160	0.053	0	15	181	0.038	0.468	1.35 (0.60–3.03)
		Meta-analysis[a]										9.83E-05	
rs2295119	T/G	GWAS	Japanese	18	139	316	0.185	41	191	284	0.265	5.77E-06	0.59 (0.47–0.74)
(class II)		Combined	Japanese	27	179	420	0.186	78	417	635	0.254	5.51E-07	0.63 (0.53–0.76)
		Replication2	Hong Kong Chinese	2	22	70	0.138	5	54	128	0.171	0.318432	0.78 (0.47–1.28)
		Replication3	Thai	4	39	136	0.131	3	50	143	0.143	0.285443	0.76 (0.47–1.25)
		Meta-analysis[a]										4.88E-07	

Table 1. Four SNPs in the HLA region associated with disease progression to HCC. [a]Results of meta-analysis were calculated by the DerSimonian-Laird method. [b]Result of logistic regression analysis adjusted for age and sex.

abolish the association in the HLA class II region but were not sufficient to abolish the association in the HLA class I region (Fig. 2 and Supplementary Material, Fig. S1f). Therefore, not only the *HLA-A* allele but also additional genetic factor(s) likely exist in the HLA class I region. There are several genes in this region including *HLA-A*, *HCG9, HLA-J, HCG8, ZNRD1-AS1, ZNRD1, PPP1R11, RNF39, TRIM31*, and *TRIM40* (shown in Fig. 2). Although these genes include pseudogenes and poorly characterized genes, some are associated with various diseases. The zinc ribbon domain-containing 1 (ZNRD1) protein is associated with cell growth of gastric cancer cells[15], angiogenesis of leukemia cells[16], and HIV-1/AIDS disease progression[17,18]. In addition, *ZNRD1* knockdown inhibits the expression of HBV mRNA and promotes the proliferation of HepG2.2.15 cells[19], suggesting that *ZNRD1* is one of the possible additional genetic factors at the HLA class I region. The tripartite motif-containing 31 (TRIM31) protein is essential for promoting lipopolysaccharide-induced Atg5/Atg7-independent autophagy[20]. Moreover, *TRIM40* is downregulated in gastrointestinal carcinomas and chronic inflammatory lesions of the gastrointestinal tract[21].

Non-self antigens, such as virus-infected cells and cancer cells, and HLA class I molecules are generally recognized by the TCRs on CD8+ T lymphocytes, resulting in T cell activation[22]. The activated T cells divide and some of their progeny differentiate into lymphocytes capable of killing cells (cytotoxic T lymphocytes: CTLs) displaying the same peptides (such as tumor-specific peptides) on their HLA class I molecules. These CTLs target tumor-specific antigenic peptides and eliminate them. In other words, CTLs cannot eliminate cancer cells without HLA class I molecules even if the person has tumor-specific peptides. Cancer cells therefore need to escape from the immune system for patients to be identified as having cancer.

In this study, we identified a significant association between *HLA-A*33:03* and HBV-related HCC. In addition to *HLA-A*33:03*, previous studies and this study suggested that *HLA-DR*, *-DQ*, and *-DP* were associated with disease progression[8,9,13]. Functional analysis of HLA class I and II proteins could be an important step in determining the pathology of HBV-related HCC.

HLA-A	Case (2n = 892)	%	Control (2n = 998)	%	Fisher's P-value	OR	95% CI
02:01	105	11.8	113	11.3	0.7733	1.04	0.78–1.40
02:06	80	9.0	106	10.6	0.2462	0.83	0.60–1.14
02:07	38	4.3	40	4.0	0.8174	1.07	0.66–1.72
11:01	53	5.9	94	9.4	0.005757	0.61	0.42–0.87
24:02	331	37.1	393	39.4	0.3198	0.91	0.75–1.10
26:01	72	8.1	89	8.9	0.5636	0.90	0.64–1.26
26:03	18	2.0	22	2.2	0.8732	0.91	0.46–1.80
31:01	112	12.6	90	9.0	0.01384	1.45	1.07–1.97
33:03	76	8.5	45	4.5	**0.00046**	**1.97**	1.33–2.95

Table 2. Association analyses of *HLA-A* alleles.

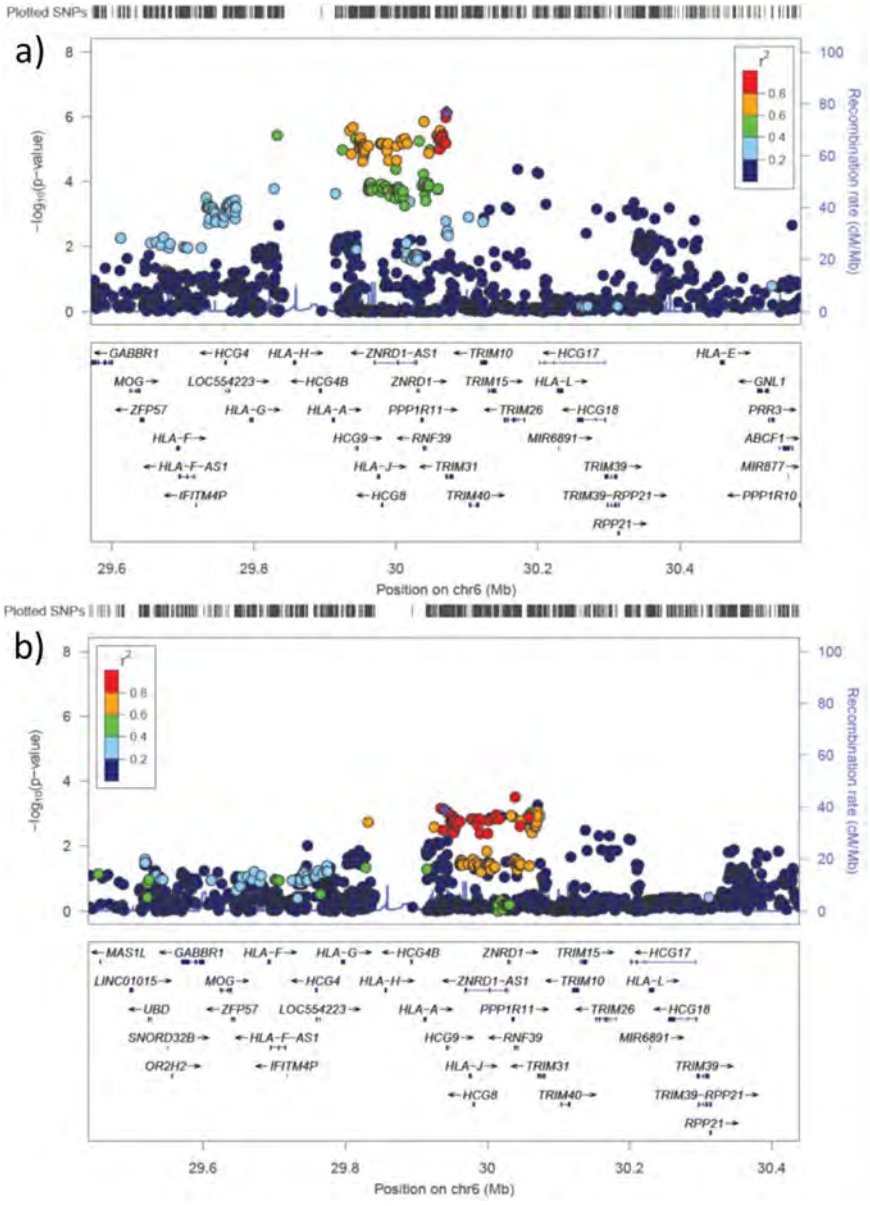

Figure 2. Association plots of the HLA class I region on chromosome 6 HLA region. (**a**) The major genetic determinant of HBV-related HCC risk to HLA class I genes. (**b**) Conditional analysis controlling for the effect of *HLA-A*33:03*.

Methods

Ethics statement. All study protocols conformed to the relevant ethical guidelines, as reflected in the *a priori* approval by the ethics committee of the University of Tokyo, and by the ethics committees of all participating universities and hospitals. All participating studies obtained informed consent from all participants in this study and all samples were anonymized.

Samples. Samples from 3,133 individuals who had HBV-derived chronic hepatitis, ASC, liver cirrhosis, or HCC and patients with other HBV-related symptoms were collected by 26 universities and hospitals (Hokkaido University Hospital, Teine Keijinkai Hospital, Iwate Medical University Hospital, Musashino Red Cross Hospital, The University of Tokyo Hospital, Saitama Medical University Hospital, Chiba University Hospital, Kitasato University Hospital, Kohnodai Hospital, Shinshu University Hospital, Kanazawa University Hospital, Nagoya City University Hospital, Kyoto Prefectural University of Medicine Hospital, National Hospital Organization Osaka National Hospital, Osaka City University Hospital, Hyogo College of Medicine, Tottori University Hospital, Ehime University Hospital, Yamaguchi University Hospital, Kawasaki Medical College Hospital, Okayama University Hospital, Nagasaki Medical Center, Kurume University Hospital, Saga University Hospital, Eguchi Hospital, and Kyusyu University Hospital). The Japanese Public Health Cancer-based Prospective (JPHC) Study samples[23] in Japan were used for the replication study. Hong Kong Chinese samples were collected at the University of Hong Kong. Thai samples were collected at Chulalongkorn University.

HBV status was measured based on serological results for HBsAg and anti-HBc with a fully automated chemiluminescent enzyme immunoassay system (Abbott ARCHITECT, Abbott Japan, Tokyo, Japan or LUMIPULSE G1200, Fujirebio, Inc., Tokyo, Japan). For clinical staging, ASC state was defined by the presence of HBsAg with normal ALT levels over 1 year (examined at least four times at 3-month intervals) and without evidence of liver cirrhosis. CH was defined by elevated ALT levels (1.5 times the upper limit of normal [35 IU/L]) persisting for over 6 months (by at least three bimonthly tests). HCC was diagnosed by ultrasonography, computerized tomography, magnetic resonance imaging, angiography, tumor biopsy, or by a combination of these.

SNP genotyping and data cleaning. For the GWAS, we genotyped 1,356 Japanese samples using the Affymetrix Axiom Genome-Wide ASI 1 Array (Affymetrix, Inc., Santa Clara, CA, USA) according to the manufacturer's instructions and determined the genotype calls of 600,307 SNPs using the Genotyping Console v4.2.0.26 software (Supplementary Material, Fig. S2a). To increase the samples for genotyping, we used not only CHB patients with and without HCC but also patients with HBV-related other symptoms such as liver cirrhosis. All samples used for genotyping passed a Dish QC >0.82 and overall call rate $>97\%$. The average Dish QC for 1,356 samples was 0.969 (0.883–0.993) and the average call rate reached 99.42% (97.47–99.87%). All genotyped samples passed a heterozygosity check, and 25 duplicated samples were identified in identity by descent (IBD) testing. A principal component analysis (PCA) found seven outliers could be excluded by the Smirnov-Grubbs test, and we showed that all the remaining samples ($n = 1,324$) formed a single cluster with the HapMap Japanese (JPT) samples but not with the Han Chinese (CHB), Northern and Western European (CEU), and Yoruban (YRI) samples. We then applied the following thresholds for SNP quality control in data cleaning: SNP call rate of $\geq 95\%$, minor allele frequency of $\geq 3\%$ and Hardy-Weinberg equilibrium P value of ≥ 0.001. A total of 447,830 SNPs on autosomal chromosomes passed the quality control filters and were used for subsequent GWAS. For the association study of HBV-related HCC, we selected 481 HBV-related HCC patients (cases) and 538 HBV carriers (CH and ASC patients, controls) from 1,324 samples and performed IBD testing and PCA again for these samples. Twenty-three related samples and seven outliers were excluded by IBD testing and PCA (Supplementary Material, Fig. S3), respectively. We finally used 473 cases and 516 controls for GWAS. A quantile-quantile plot of the distribution of test statistics for the comparison of genotype frequencies in the cases and controls showed that the inflation factor λ was 1.016 for all tested SNPs and was 1.009 when SNPs in the HLA region were excluded (Supplementary Material, Fig. S4). All cluster plots for SNPs with P values of $<10^{-4}$ were checked visually and SNPs with ambiguous genotype calls were excluded.

In the replication stage, we selected seven SNPs with P values of $<10^{-5}$ from the results of the chi-square test in the GWAS. A TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) was used to confirm the genotypes at each SNP. We genotyped 989 and 767 Japanese samples for the validation of the GWAS and for the replication study, respectively. We further genotyped 281 Hong Kong Chinese and 383 Thai samples for the replication study (Supplementary Materials, Table S3).

Statistical analysis. The characteristics of analyzed samples are shown in Supplementary Materials, Table S3. For the GWAS and replication study, the chi-square test was applied to a two-by-two contingency table in the allele frequency model. Meta-analysis was performed using the DerSimonian-Laird method (random-effects model) in order to calculate the pooled OR and its 95% confidence interval. Fisher's exact test in a two-by-two contingency table was used to examine the association between *HLA* alleles and disease progression of HBV patients. To avoid false-positive results due to multiple testing, the resulting P-values were adjusted based on the number of observed alleles with frequencies $\geq 0.5\%$ in cases and controls. Conditional logistic regression analysis was performed for SNPs and *HLA* alleles. This analysis was performed as implemented in Plink v1.07 software[24], conditioning on *HLA-A*33:03* and *DPB1*02:01* to each of the other SNPs. Other statistical analyses were performed using the SNP & Variation Suite 7 software (Golden Helix, Bozeman, MT, USA) and statistical software R v2.6. Manhattan plot of conditioning of each SNP or HLA allele was generated by LocusZoom[25].

HLA imputation. SNP data from 989 samples were extracted from extended MHC (xMHC) regions ranging from 25759242 bp to 33534827 bp based on hg19 position. Two-field HLA genotype imputation was performed for a total of six HLA class I and class II genes using the HIBAG R package[26,27]. For *HLA-A,-B, -DRB1, -DQB1*,

and -*DPB1*, a Japanese imputation reference[26] was used for HLA genotype imputation. For *HLA-C*, the HIBAG Asian reference[27] was used for HLA genotype imputation. We applied post-imputation quality control using call-threshold (CT > 0.5); the call rate of successfully imputed samples ranged from 88.7 to 98.5% for the six HLA classes. In total, we imputed 5,650 HLA genotypes in HLA class I and class II genes.

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a p value<0.001. The factors associated with bullying were the younger age group, shorter length of service, shifting work, non-managerial position and the designation as a doctor.

Conclusion A significant proportion of healthcare workers had been bullied, and bullying exposure was shown to be associated with depression and low self-esteem. Hence, regular screening for bullying, depression and low self-esteem should be done to enable early intervention.

1551 CHANGES IN TWENTY YEARS OF THE EPIDEMIOLOGICAL STATUS OF NEEDLESTICK/SHARPS INJURIES REPORTED TO JAPAN-EPINET THROUGH A NATION-WIDE SURVEILLANCE NETWORK

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Introduction This study aimed at examining annual logs of needlestick/sharps injuries (NSIs) collected through a voluntary nation-wide surveillance network in twenty-years for preventing occupational blood-borne infections. The emphasis was placed on revealing the past and current situations of NSIs in health care settings.

Methods Japan-EPINet format was developed by the technical support of the International Healthcare Worker Safety Centre, University of Virginia in the United States in 1996. Japan-EPINet Surveillance (JES) was conducted by the Research Group for Occupational Infection Control and Prevention in Japan (JRGOICP). Data were analysed in four phases of the nation-wide surveillance network of AIDS referral hospitals out of a total of 364 registered, a total number of hospital-year was 1879. These hospitals reported employees' percutaneous injuries on a voluntary basis.

Results A total of 65,032 NSIs were reported to Japan-EPINet from 1996 to 2015. The rate of hepatitis C antibody positive cases of the total NSIs decreased from 69.9% (1,511/2,161) in 1996 to 11.5% (714/6,201) in JES2015. The proportion of NSIs due to 'recapping' decreased (28.7%, 6.9% respectively). Devices caused to NSIs by winged steel needles (25.3%, 8.6%) and vacuum tube phlebotomy needles (4.8%, 1.7%) were decreased, disposal syringe (28.5%, 26.2%) and IV catheter (6.7%, 5.2%) were fairly decreased. The proportion of Suture needle (10.3%, 16.9%) and pre-filled cartridge syringe (2.8%, 8.3%) were increased.

Discussion The changes of characteristics NSIs in Japan in twenty-year suggested that recognition of the risks of NSIs was vital for promoting the effective use of safety-engineered needle/sharp devices and point-of-use disposal containers because the rate of hepatitis C antibody positive cases among voluntary reported NSIs. The creation of the nation-wide surveillance network was effective for monitoring and evaluating NSIs and for focusing on implementation of effective countermeasures.

25 PREPARATION OF HAZARDOUS DRUGS IN BIOLOGICAL SAFETY CABIN (BSC): THE CHALLENGE OF GETTING HEALTHIER WORK ENVIRONMENTS

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Introduction Hazardous drugs are an important risk to health care workers. Some of these products may even be potentially carcinogenic.

In different Spanish hospitals it was observed that only Cytostatics drugs were prepared in biological safety cabins, leaving workers exposed to the rest of hazardous non cytostatic drugs.

Methods A bibliographical review of scientific articles and researches has been carried out, together with the laws on occupational health and recommendations of the Spanish organisms.

In the USA, research promoted the development of policies of prevention and the incorporation of these drugs in the list NIOSH.

Result After analysing the information obtained, we detected the following problems: HD's are prepared in hospitalisation rooms, where the right conditions to protect workers are non-existent; In many cases, health care workers are given only personal protective equipment to avoid exposure; Specific health control isn't performed in most cases; National legislation obliges the risk to be taken into account for the worker. Although there are no long-term epidemiological studies, protective measures should be taken.

Discussion In many hospitals in our country HD's are not prepared in biological safety cabins. Health workers are unaware that they are exposed to these risks and no specific health training or monitoring is performed. Collaborative epidemiological researches should be promoted among Public Health Units, which have information on the prevalence rate of cancer diseases, and those responsible for occupational health prevention.

250 HOW THE WORKING BACKS PROGRAMME HELPED STAFF MANAGE BACK PAIN, REMAIN IN WORK AND REDUCE ABSENTEEISM

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Introduction The Working Backs Programme (WBP) is designed for staff reporting back pain as a result of work or whose work performance is affected. It's a comprehensive approach including medical assessment, provision of information and education, a designated physiotherapy and ergonomic staff referral service and a referral pathway for further investigations and/or review. The effectiveness was evaluated by an initial audit in 2012 and subsequent audits in 2015 and 2016.

Methods Data was collected through questionnaires at initial consultation and post discharge for comparison. This included

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＜特別寄稿＞

日本肝臓学会評議員を対象としたB型肝炎ワクチンに関するアンケート調査

田中　靖人[1)]　乾　あやの[2)]　森屋　恭爾[3)]　江口有一郎[4)]　四柳　宏[5)]

要旨：B型肝炎（HB）ワクチンの在り方を検討するために，日本肝臓学会HBワクチンワーキンググループとして日本肝臓学会評議員などを対象にHBワクチンに関するアンケート調査を実施した．その結果，1)「HBワクチンの適切な接種時期（キャッチアップ）」に関しては，小学生高学年64%と最多であった．2)「ワクチン無効例に対する対策」としては，筋肉内注射や4回以上投与などが挙げられた．3)「HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性」について，「必要」が63%で最も多く，その施設の多くは職員に対するHBs抗体の定期検査を12カ月ごとに行い，HBs抗体価10 mIU/mL未満の時点でHBワクチンを追加接種していた．これらの結果を踏まえると，「追加のワクチン接種は必要ではない」とする日本環境感染学会ガイドラインについて再度議論する必要があるように思われた．

索引用語：　HBV　B型肝炎ワクチン　ワクチンブースター　HBs抗体

緒　言

わが国では，1972年に日本赤十字社の血液センターにおけるHBs抗原のスクリーニング検査が開始された．さらに，1986年に開始された母子感染防止事業に基づく出生児に対するワクチンおよび免疫グロブリン投与により，垂直感染による新たなHBVキャリア成立が阻止され，若年者におけるHBs抗原陽性率は著しく減少した．しかし，一方で性交渉に伴う水平感染によるB型急性肝炎の発症数は減少せず，近年では，肝炎が遷延し慢性化しやすいゲノタイプAのHBV感染が増加傾向にある[1)]．

2016年10月より0歳児を対象としたB型肝炎（HB）ワクチンの定期接種が開始されたが，定期接種の対象から漏れた小児への対応，性行為感染症としてのB型急性肝炎，ワクチン無反応・低反応者対策，ブースター接種の必要性，HBワクチン接種によるHBV再活性化抑制などの問題が残されている．

また，HBVワクチン接種によって免疫が得られても，HBs抗体は最初の1年で急速に低下し，それ以降はゆっくりと減少する．健常人では，ワクチン接種者の90～95%に抗体産生がみられるが，抗体産生は時間の経過とともに減弱し，8年以上経過すると約60%の人で抗体が検出されなくなる．しかし，HBVに対する免疫は保たれるため，再度ワクチンを接種する必要はないとしている[2)3)]．実際，4～23年前にワクチンが接種されてHBs抗体を獲得したにも拘わらず，時間の経過によって10 mIU/mL未満まで低下してしまった人にワクチンをブースター接種すると僅か2～4週間後に74～100%の人で抗体が再陽転化した．このデータはワクチン接種者の多くが免疫記憶を維持しており，HBVの曝露によってHBs抗体を獲得することができることを示している．以上の結果を踏まえて，米国CDC（Centers for Disease Control and Prevention）ガイドラインでは，一度十分な抗体価が得られれば，その後抗体価が低下しても曝露に際して効果的な免疫反応が得られると判断され，腎不全を含む免疫不全症例以外は，経時的な抗体価測定は不要とした[4)]．

今回，HBワクチンの在り方を検討するために，小池和彦理事長の承認の下，企画広報委員会（持田　智委員長）に依頼して，同委員会内にHBワクチン小委員会を設置し，日本肝臓学会HBワクチンワーキンググループ（WG）として日本肝臓学会評議員などを対象にHBワクチンに関するアンケート調査を実施したので，その結果を報告する．

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Table 1　B型肝炎ワクチンに関するアンケートの様式

B型肝炎ワクチンに関するアンケートのお願い

一般社団法人　日本肝臓学会
企画広報委員会　委員長　持田智
HBワクチン小委員会

2016年10月より0歳児を対象としたB型肝炎(HB)ワクチンの定期接種が開始されました。現在残された問題点として、定期接種の対象から漏れた小児への対応、性行為感染症としてのB型急性肝炎（欧米型A）及びHBV再活性化があり、これらの点に関して学会として対応を考えるべく、「HBワクチン小委員会」が発足致しました。つきましては今回、日本肝臓学会評議員の先生方のご意見を伺いたく簡単なアンケートを実施させて頂きますので、以下の質問に対する御回答をお願いします。**いずれも複数回答可です。**

1．定期接種の対象とならなかった人に対するキャッチアップとしてHBワクチンの適切な時期についてお尋ねします。
　□ 小学生高学年（他のワクチンと同時接種）
　□ 中学生　　□ 高校生
　□ キャッチアップ必要なし

2．ワクチン無効例に対する対策はどのようにされていますか？これまでの報告（八橋弘 B型肝炎ワクチンの筋肉内注射. 日本医事新報 4858:53-58, 2012）によると筋肉内注射により有意なHBs抗体価上昇が期待できます。
　（接種方法の変更）□ 筋肉内注射　　□ 皮内注射
　□ ワクチンの種類を変更　　□ 倍量投与　　□ 4回以上投与
　□ その他（　　）

3．院内で、職員に対するHBs抗体の採血は定期的にされていますか？
　□ はい　　□ いいえ
　「はい」の場合の頻度（　　）ヶ月おき

4．HBs抗体価が低下した医療従事者に対するHBワクチンのブースターはされていますか？　　□ はい　　□ いいえ
　「はい」の場合の目安
　□ HBs抗体 10 mIU/mL未満（陰性）　　□ HBs抗体100 mIU/未満

5．その他、ご意見がございしましたら、よろしくお願いします。

方　法

平成29年9月, 日本肝臓学会HBワクチンワーキンググループとして日本肝臓学会評議員など855名を対象にTable 1のようなアンケート調査を実施した. 1)定期接種の対象とならなかった人に対するキャッチアップとしてHBワクチンの適切な接種時期, 2)ワクチン無効例に対する対策, 3)院内職員に対するHBs抗体の定期検査の実施状況, 4)HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性と実際の対応について質問した.

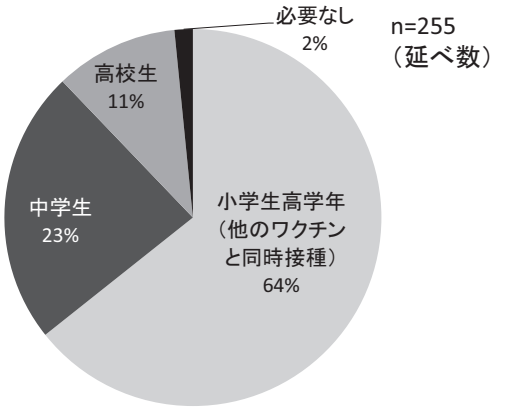

Fig. 1　HBワクチンの適切な接種時期（キャッチアップ）

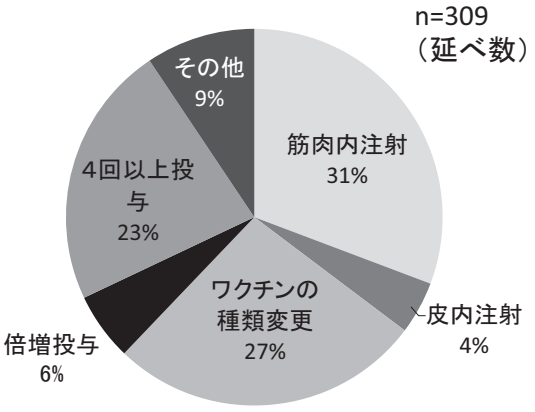

Fig. 2　ワクチン無効例対策

結　果

アンケート調査の回収率は24%（206/805）であった．1）「HBワクチンの適切な接種時期（キャッチアップ）」に関しては，小学生高学年（他のワクチンと同時接種）64%，中学生23%，高校生11%であった（Fig. 1）．2）「ワクチン無効例に対する対策」としては，筋肉内注射31%（皮内注射4%），ワクチンの種類を変更27%，4回以上投与23%，倍量投与6%であった（Fig. 2）．3）「職員に対するHBs抗体の定期検査の有無」は，「あり」62%で，検査頻度は12カ月毎の採血が91%と最多であった（Fig. 3）．4）「HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性」について，「必要」63%で，このうち93%でHBs抗体価10 mIU/mL未満の時点で実施していた（Fig. 4）．

考　察

米国CDCガイドラインの発表を受けて，日本環境感染学会ガイドラインでも「ワクチン接種シリーズ後の抗体検査で免疫獲得と確認された場合，その後の抗体検査や追加のワクチン接種は必要ではない」という勧告を出した[5]．すなわち，1）透析患者，2）HIV感染者，3）造血幹細胞移植を受けた患者，4）化学療法や免疫抑制療法を受けた患者などのハイリスクグループ以外は追加のワクチン接種は必要ではないとするガイドラインである．確かに，集団免疫（医療機関として）の観点からは，医療従事者の肝炎発症と患者への2次感染を防ぐことが目標であり，コストベネフィットを考慮した米国のガイドラインは正しいと言えよう．

一方，個人免疫の観点からは肝炎も嫌だが，将来の肝がんも防ぎたい．すなわち，HBc抗体が陽性化する感染を防ぐことにより，肝炎，肝癌，さらにはHBV再活性化すべてを予防することが可能となる．実際に福祉の国であるイギリスのガイドラインでは，抗体低下時の追加接種を推奨しており，HBs抗体価10～100 mIU/mLの人でさえ，1回追加接種したのち5年ごとに1回追加接種を推奨している[6]．特に，1）医療従事者，2）透析患者，3）パートナーや家族内にHBVキャリアがいる場合は強く推奨される．興味深いことに，今回の日本肝臓学会評議員などを対象としたアンケート調査では，「HBs抗体価が低下した医療従事者に対するHBワクチンのブースターの必要性」について，「必要」が63%で最も多く，その施設の多くは職員に対するHBs抗体の定期検査を12カ月ごとに行い，HBs抗体価10 mIU/mL未満の時点でHBワクチンを追加接種していた．これらの結果を踏まえると，「追加のワクチン接種は必要ではない」とする日本環境感染学会ガイドラインについて再度議論する必要があるように思われる．これは“B型肝炎”を「肝臓病」として捉えている肝臓専門医と「感染症」として捉えている感染症専門医との間にある根本的な考え方の相違に起因するものかもしれない．

これまでに医療従事者を何百人も対象とした研究や男性同性愛者やエスキモーを対象とした研究が長期間実施されており，これらの研究の成果はCDCからの勧告を支持しているが，HBc抗体が検出された症例が存在するのも事実である[7)～9)]．HBc抗体はHBVワクチンでは獲得されない抗体であり，この存在はHBV自体が体内に入り込み，免疫が反応したという根拠になる．すなわち，HBワクチン接種でHBs抗体陽性となった場合，その後のHBVへの曝露により肝炎を発症するこ

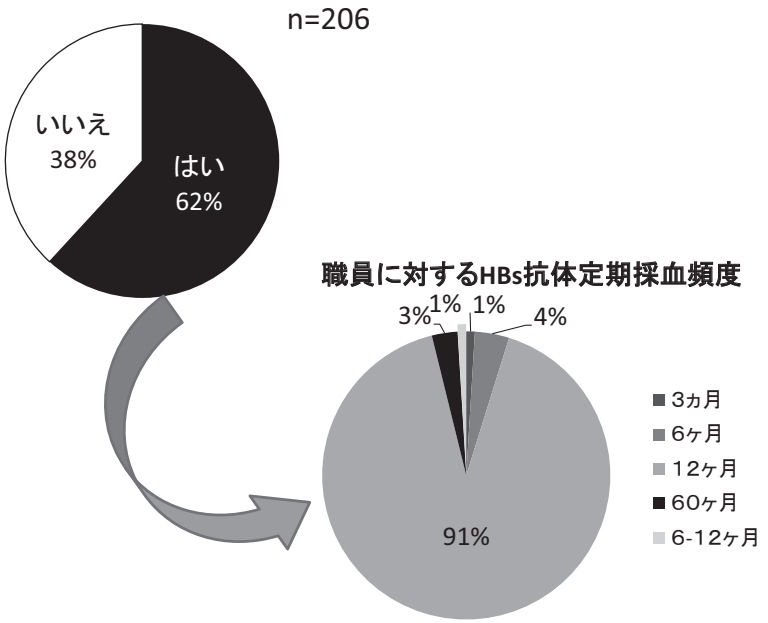

Fig. 3　職員に対する HBs 抗体の定期採血

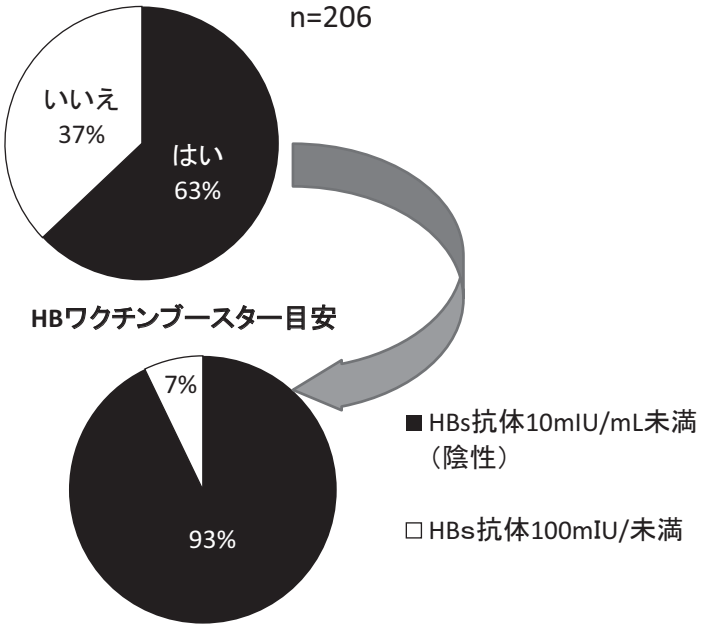

Fig. 4　医療従事者に対する HB ワクチンブースター

とはまれであるが，HBs 抗体価が低下した際には HBV への曝露後に HBV DNA が陽性となることがある[10]．このような状態はオカルト HBV 感染と称され，免疫抑制状態において HBV 再活性化を引き起こすことがある[11]．現在のところ，HB ワクチン接種後 HBs 抗体が陰転化した場合の HB ワクチン追加接種は推奨されていないが，HB ワクチン接種数年後に HBs 抗体価が低下し，急性肝炎（ALT 3,510 U/L）を発症した症例[12]や急性肝

炎発症（ALT 211 U/L）からキャリア化した症例[13]も報告されており，HBs抗体価10 mIU/mL未満に低下した場合にはHBワクチンを追加接種することも選択肢となりうる．特に，肝炎を発症しないまでも，HBc抗体が陽転化した時点で，肝臓内にはHBVはすでに侵入・感染していることになり，がん化学療法や免疫抑制剤使用時にHBV再活性化のリスクを背負うことになる．そのような予測可能な事態を肝臓専門医として容認してよいのか，今後も議論が必要と思われる．

結　語

日本肝臓学会評議員などを対象にアンケート調査を行った結果，HBワクチンに関する重要なエクスパートオピニオンが得られた．今後も，日本肝臓学会としての意見をまとめて広く情報発信する予定である．

謝辞：今回，HBワクチンの在り方を検討するための「日本肝臓学会HBワクチンワーキンググループ（企画広報委員会HBワクチン小委員会）」設立にご尽力頂きました小池和彦理事長ならびに企画広報委員会委員長の持田智先生に深く感謝申し上げます．なお，本アンケートにご協力いただきました日本肝臓学会役員及び評議員の先生方に深謝いたします．

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ORIGINAL ARTICLĒLIVER, PANCREAS, AND BILIARY TRACT

Epidemiologic features of 348 children with hepatitis C virus infection over a 30-year period: a nationwide survey in Japan

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Abstract

Background Although the epidemiology of hepatitis C virus (HCV) infection among children may be rapidly changing, few reports have characterized large nationwide cohorts of children with HCV infection. We, therefore, sought to clarify the epidemiology and natural history of HCV infection in Japanese children born over the last three decades.

Methods Sixty-five pediatric centers retrospectively and prospectively recruited consecutive, otherwise-healthy HCV-infected children born during 1986 to 2015.

Results Entry criteria were met by 348 children. Age at initial diagnosis of infection has decreased significantly in recent years. Cirrhosis and hepatocellular carcinoma were not identified. Prevalence of spontaneous clearance and of interferon treatment with/without ribavirin were 9 and 54%, respectively. Maternal transmission has increased significantly, representing over 99% of cases in the last decade. No transfusion-related cases have been seen after 1994. HCV genotype 2 has increased to become the most prevalent in Japanese children. Histopathology examination of liver specimens showed no or mild fibrosis in most children with chronic hepatitis C; none showed cirrhosis.

Conclusions This largest nationwide cohort study of Asian children with HCV infection spanned the last three decades. None of these Japanese children developed cirrhosis or hepatocellular carcinoma. Maternal transmission increased to account for 99% of cases during the last decade. Genotype 2 now is most prevalent in these children. Histopathologically, most children with chronic hepatitis C showed mild fibrosis or none.

Keywords Natural history · Maternal transmission · Genotype · Liver histopathology · Cirrhosis

Abbreviations

HCV	Hepatitis C virus
anti-HCV	Anti-HCV antibody
SVR	Sustained virologic response
IFN	Interferon
RBV	Ribavirin
CHC	Chronic hepatitis C
SD	Standard deviation
DAAs	Direct-acting antiviral agents

Introduction

Hepatitis C virus (HCV) infection is a major cause of liver disease. Recent estimates showed an increase in its worldwide prevalence over the last decade to 2.8%, amounting to

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over 185 million infections [1–3]. In Japan, estimated prevalence of HCV infection in adults has been 0.8 to 1.2% [4]. Prevalence is lower in children, estimated at 0.012% at ages 5–9 years, 0.010% at 10–14 years, and 0.022% at 15–19 years [5]. The low prevalence of HCV infection in children reflects disappearance of transmission by blood transfusions and other medical procedures, and also reduced mother-to-child (i.e., vertical or perinatal) transmission, even though this form of transmission currently is responsible for most new infections in developed countries [6–9]. Among HCV genotypes, genotype 1 is most prevalent worldwide (49.1%), followed by genotypes 3 (17.9%), 4 (16.8%), and 2 (11.0%). Genotypes 5 and 6 are responsible for the remaining infections, representing less than 5% [3]. In Japanese adults, relative prevalence of genotype 1 has declined while that of genotype 2 has increased; nonetheless, genotype 1 (65%) remains more prevalent than genotype 2 (34%) [4, 10]. Taken together, these data raise the question of possible rapid changes in the epidemiology of HCV infection among Japanese children, but few large nationwide cohort studies of children with HCV infection have been undertaken, particularly in the last decade [9, 11, 12]. To evaluate the extent of these changes, which could alter the future burden of HCV infection, we investigated epidemiologic features of a large nationwide cohort of children with HCV infection in Japan. Specifically, we aimed to clarify the epidemiology and natural history of HCV infection in Japanese children who were born over the last three decades.

Methods

Study design

This study was designed and conducted within the framework of the "Observatory for HCV Infection and Hepatitis C in Japanese Children," established in 2011 by the Hepatology Group of the Japanese Society for Pediatric Gastroenterology, Hepatology and Nutrition (JSPGHAN) with the aim of taking a census of children with HCV infection and investigating clinical aspects and outcomes of liver disease in this inadequately studied population. Sixty-five pediatric centers in Japan were involved in this survey. Over approximately 4 years, each of these centers retrospectively and prospectively collected all anti-HCV antibody (anti-HCV)-positive cases in children born from 1986 to 2015. Baseline and follow-up clinical information were obtained from patient records. Patient characteristics, clinical diagnosis at last visit, treatment, type of exposure, HCV genotype, and histopathologic features of liver biopsy specimens were determined. Features of the patients were evaluated in three groups defined by birth year: 1986–1995, 1996–2005, and 2006–2015. Some of these patients have been involved in previous studies [12–14]. The study protocol complied with the ethical guidelines of the Declaration of Helsinki of 1975 (2004 revision) and was approved by the ethics committee of Osaka General Medical Center and other participating centers.

Patients

Inclusion criteria were age between 0 and 16 years at initial diagnosis, birth between 1986 and 2015, HCV RNA positivity in at least one serum sample, follow-up for at least 1 year after the infection was diagnosed at the observatory center, and absence of coinfection with human immunodeficiency virus (HIV) or hepatitis B virus (HBV).

Clinical definitions were as follows. Spontaneous sustained clearance (in untreated HCV RNA-positive patients) signified disappearance of HCV RNA from at least two consecutive serum samples. Carriers were HCV RNA-positive patients with persistently normal serum alanine aminotransferase (ALT) concentrations. Chronic hepatitis was diagnosed in HCV RNA-positive patients with persistently increased ALT for more than 6 months or a liver biopsy specimen showing chronic hepatitis. Sustained virologic response (SVR) indicated HCV RNA negativity for 24 weeks following conclusion of interferon (IFN) treatment with/without ribavirin (RBV). Evidence of cirrhosis was diagnosed by liver biopsy or by clinical findings (jaundice, fatigue and/or edema), blood tests (hyperbilirubinemia, thrombocytopenia, hypoalbuminemia, and/or coagulopathy), and/or abdominal imaging including the liver using ultrasonography, computed tomography and/or magnetic resonance imaging (ascites, nodularity of the liver, and/or atrophy of the liver).

Type of HCV exposure

Putative types of HCV exposure were evaluated by concordant results of HCV genotype between mother and child and by ascertaining family history and past surgical and transfusion histories.

HCV RNA and genotype

HCV RNA was quantified in fresh or well-preserved stored sera by commercial quantitative assays such as real-time PCR (COBAS Ampliprep/COBAS TaqMan HCV test, Roche) in 90% of subjects, amplicor HCV monitor (COBAS Amplicor HCV Monitor test v 2.0, Roche) in 8% and branched DNA probe (Quantiplex HCV RNA 2.0, Bayer) in 2%. Genotype was assessed by genotyping assay using reverse transcription PCR of the core region with the genotype-specific primers in 82% of subjects and by serotyping assay in 18% according to the international classification [15, 16].

Histopathology

Histopathology of the liver was evaluated using initial liver biopsy specimens obtained from children with chronic hepatitis C (CHC) before they had received any IFN treatment with/without RBV. Liver biopsy specimens were assessed pathologically based on the New Inuyama Classification of chronic hepatitis [17], in which chronic hepatic disease is characterized according to degree of fibrosis (F) as follows: F0 (no fibrosis, equivalent to Ishak stage 0), F1 (fibrosis evident as portal expansion, equivalent to Ishak stage 1–2), F2 (bridging fibrosis, equivalent to Ishak stage 3), F3 (bridging fibrosis with lobular distortion, equivalent to Ishak stage 4), or F4 (cirrhosis, equivalent to Ishak stage 5–6) [17, 18]. Additionally, the classification assesses chronic hepatic disease activity (A) based on degree of lymphocytic infiltration and necrosis of hepatocytes as follows: A0 (no necro-inflammatory reaction), A1 (mild necro-inflammatory reaction), A2 (moderate necro-inflammatory reaction), and A3 (severe necro-inflammatory reaction) [17].

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) and categorical variables as frequencies and percentages. Chi squared, Fisher's exact, ANOVA, Tukey–Kramer, and Pearson correlation tests were used as appropriate. All statistical analysis was performed using GraphPad Prism version 6.05 software (GraphPad Software, San Diego, CA, USA). Tests were two-sided. *P* values below 0.05 were considered to indicate statistical significance.

Results

During this survey, participating centers enrolled 441 consecutive anti-HCV-positive children, among whom 348 children met entry criteria. Based on birth year, they were assigned to one of three groups: group 1, including 49 children born between 1986 and 1995; group 2, including 175 born between 1996 and 2005; or group 3, including 124 born between 2006 and 2015 (Fig. 1). Ninety-three children were excluded from this study for the reasons such as unknown RNA positivity, follow-up for less than 1 year, or presence of coinfection with HIV or HBV.

Patient features

Table 1 summarizes distribution of gender, age at initial diagnosis of infection, age at last clinical visit, clinical diagnosis at last visit, and treatment in the three groups.

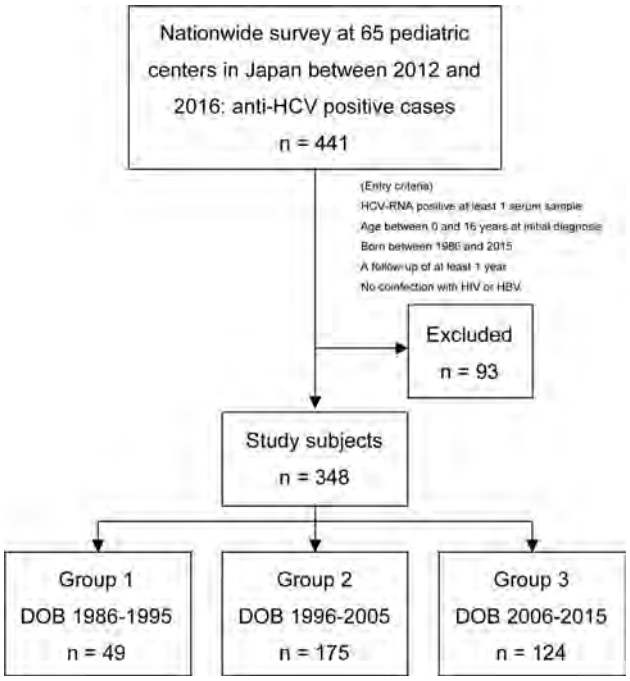

Fig. 1 Flow chart of this study. This chart summarizes entry criteria and distribution of patients into groups according to birth year. *HCV* hepatitis C virus, *anti-HCV* anti-HCV antibody, *n* number of patients, *HIV* human immunodeficiency virus, *HBV* hepatitis B virus, *DOB* date of birth

Girls accounted for 56% of patients. Age at initial diagnosis of infection had decreased significantly in recent years ($P < 0.0001$). As for clinical diagnosis at last visit, frequencies of spontaneous clearance, carrier state, chronic hepatitis, and SVR were 9, 34, 4, and 40%, respectively. Carriers had increased significantly in recent years ($P < 0.0001$), and SVR had decreased significantly ($P < 0.0001$). Cirrhosis and hepatocellular carcinoma were not identified. The overall fraction of patients who received IFN treatment with/without RBV in recent years was 54%, having decreased significantly ($P < 0.0001$).

Type of HCV exposure

Table 2 characterizes the 348 children based on putative type of exposure to HCV in the three groups. Maternal transmission, the most frequent source of infection in all groups, accounted for 90% of infections overall, with a significant increase in recent years ($P < 0.0001$), increasing to over 99% in the last decade. Transfusion was the second most frequent source of infection in the earliest decade, while no transfusion-related cases have been seen since 1994. Only 17 cases (5%) were ascribed to other putative sources of infection, horizontal transmission or unknown source.

Table 1 Demographic and clinical features of the 348 children enrolled in the study

	Total ($n = 348$)	Group 1 1986–1995 ($n = 49$)	Group 2 1996–2005 ($n = 175$)	Group 3 2006–2015 ($n = 124$)	P values[a]
Male, n (%)	154 (44)	21 (43)	79 (45)	54 (44)	0.9418
Age at diagnosis of infection, months[b,f]	37.7 ± 45.2	76.7 ± 59.6	43.0 ± 44.1	13.0 ± 16.0	<0.0001
Age at last visit, months[b,f]	130.7 ± 70.2	240.6 ± 49.6	148.9 ± 38.0	61.7 ± 28.8	<0.0001
Clinical diagnosis at last visit, n (%)					
Spontaneous clearance	30 (9)	1 (2)	13 (8)	16 (13)	0.0525
Carrier[c]	120 (34)	9 (19)	45 (26)	66 (53)	<0.0001
Chronic hepatitis	15 (4)	1 (2)	6 (3)	8 (6)	0.3134
Sustained virologic response[d]	139 (40)	33 (67)	88 (50)	18 (15)	<0.0001
During treatment	16 (5)	1 (2)	9 (5)	6 (5)	0.6488
Unknown	28 (8)	4 (8)	14 (8)	10 (8)	0.9993
Cirrhosis/HCC	0/0				
Treatment (IFN with/without RBV), n (%)[e]	188 (54)	37 (76)	118 (67)	33 (27)	<0.0001

n number of patients, *HCC* hepatocellular carcinoma, *IFN* interferon, *RBV* ribavirin

[a] Comparison among the 3 groups by Chi squared or ANOVA tests

[b] $P < 0.0001$, Group 1 vs. Group 2, Group 1 vs. Group 3, and Group 2 vs. Group 3 by Tukey–Kramer test

[c] $P < 0.0001$, Group 1 vs. Group 3 and Group 2 vs. Group 3 by Fisher's exact test

[d] $P = 0.0364$, Group 1 vs. Group 2; $P < 0.0001$, Group 1 vs. Group 3 and Group 2 vs. Group 3 by Fisher's exact test

[e] $P < 0.0001$, Group 1 vs. Group 3 and Group 2 vs. Group 3 by Fisher's exact test

[f] Mean ± standard deviation

Table 2 Putative types of exposure to HCV infection in 348 children

	Total ($n = 348$)	Group 1 1986–1995 ($n = 49$)	Group 2 1996–2005 ($n = 175$)	Group 3 2006–2015 ($n = 124$)	P values[a]
Maternal, n (%)[b]	314 (90)	30 (61)	161 (92)	123 (99)	<0.0001
Horizontal, n (%)	2 (1)	0	2 (1)	0	0.3700
Transfusion, n (%)[c]	17 (5)	17 (35)	0	0	<0.0001
Unknown, n (%)[d]	15 (4)	2 (4)	12 (7)	1 (1)	0.0398

n number of patients

[a] Comparison among the three groups by Chi squared test

[b] $P < 0.0001$, Group 1 vs. Group 2 and Group 1 vs. Group 3; $P = 0.0054$, Group 2 vs. Group 3 by Fisher's exact test

[c] $P < 0.0001$, Group 1 vs. Group 2 and Group 1 vs. Group 3 by Fisher's exact test

[d] $P = 0.0176$, Group 2 vs. Group 3 by Fisher's exact test

HCV genotype

Table 3 characterizes 298 of the children based on the HCV genotypes in the three groups. Overall relative prevalences of genotypes 1, 2, and 3 were 42, 57, and 1%, respectively. Genotype 1 has decreased significantly in recent years ($P = 0.0427$), while genotype 2 has increased ($P = 0.0775$).

Histopathology

Table 4 summarizes the demographic and clinical features of 147 children with CHC who underwent liver biopsy between 1995 and 2015, while Table 5 presents the histopathologic features of the liver according to the New Inuyama Classification [17]. Mean age at biopsy was 8.9 ± 4.0 years. The distribution of degree of necro-

Table 3 HCV genotype in 298 children

	Total ($n = 298$)	Group 1 1986–1995 ($n = 44$)	Group 2 1996–2005 ($n = 158$)	Group 3 2006–2015 ($n = 96$)	P values[a]
Genotype 1, n (%)[b]	126 (42)	25 (57)	68 (43)	33 (34)	0.0427
Genotype 2, n (%)	169 (57)	19 (43)	89 (56)	61 (64)	0.0775
Genotype 3, n (%)	3 (1)	0	1 (1)	2 (2)	0.4095

n number of patients

[a] Comparison among the three groups by Chi squared test

[b] $P = 0.0162$, Group 1 vs. Group 3 by Fisher's exact test

Table 4 Demographic and clinical features of 147 children with chronic hepatitis C who underwent liver biopsy between 1995 and 2015

Male, n (%)	70 (48)
Age at biopsy, years[a]	8.9 ± 4.0
Duration of infection, years[a] (maternal transmission, $n = 127$)	8.4 ± 3.6
Type of exposure, n (%)	
Maternal	127 (86)
Transfusion	10 (7)
Horizontal or unknown	10 (7)
HCV genotype ($n = 131$), n (%)	
Genotype 1	63 (48)
Genotype 2	66 (50)
Genotype 3	2 (2)

n number of patients

[a] Mean ± standard deviation

Table 5 Histopathologic features of liver biopsy specimens from 147 children with chronic hepatitis C

N (%)	A0 (5)	A1 (74)	A2 (20)	A3 (1)
F0 (33)	6	34	8	0
F1 (58)	2	70	12	1
F2 (9)	0	5	9	0

n number of patients, *A0* no necro-inflammatory reaction, *A1* mild necro-inflammatory reaction, *A2* moderate necro-inflammatory reaction, *A3* severe necro-inflammatory reaction, *F0* no fibrosis, *F1* fibrosis with portal expansion, *F2* bridging fibrosis

inflammatory activity (A0, A1, A2, and A3) was 5, 74, 20, and 1%, respectively. The distribution of degree of fibrosis (F0, F1, and F2) was 33, 58, and 9%, respectively. F3 and F4 were not seen. No significant correlation was found between degree of fibrosis and age at biopsy or duration of infection (Supplementary Figs. 1 and 2). Degree of fibrosis was not related to gender, type of exposure, or genotype (Supplementary Tables 1 to 3).

Discussion

Few reports describing large nationwide cohorts of children with HCV infection are available, although recent reports concerning adults indicate that the epidemiology of HCV infection is changing dramatically worldwide [1–3, 9, 11, 12]. We investigated the epidemiologic features of Japanese children with HCV infection to clarify natural history and trends over the last three decades. Previous large nationwide cohort studies of children with HCV infection describe epidemiologic features observed about two decades before 2006 [9, 11, 12]. Our investigation represents the largest nationwide cohort study of Asian children with HCV infection over a 30-year period, including children born during the most recent decade, 2006–2015. Additionally, we included a large pediatric-age survey of HCV histopathologic features, characterizing 147 children with CHC.

Since HCV was discovered in 1989 [19, 20], the Japanese Red Cross has screened blood donors for anti-HCV with a first-generation assay beginning in 1989, or, since 1992, a second-generation assay [21]. The present study shows that because of screening, transfusion transmission has decreased dramatically, and transfusion-related cases have disappeared after 1994. Three patients had putative transfusion transmission between 1992 and 1994, most likely because risk of fibrinogen-transmitted HCV infection was yet to be eliminated in Japan during that period [22]. At present maternal transmission accounts for 99% of cases, representing nearly the sole route for pediatric-age HCV infection. Comparing group 2 (born from 1996 to 2005) with group 3 (2006–2015), ages at time of diagnosis steadily decreased. We believe that this change reflects heightened awareness of maternal transmission of HCV among Japanese obstetricians and pediatricians; nearly all pregnant women in Japan now are screened for anti-HCV.

Girls were somewhat more numerous than boys among our subjects (56%) and spontaneous clearance occurred in 9% of patients, in essential agreement with previous reports [9, 11, 23]. IFN treatment with/without RBV was given to 54% of patients. Suzuki et al. reported that pegylated IFN monotherapy and pegylated IFN combined with RBV both produced encouraging results against HCV infection and were well tolerated and reasonably safe in Japanese children and adolescents with CHC, including some enrolled in this study [13]. Interestingly, our survey identified no patients with cirrhosis. Bortolotti et al. reported that 2% of untreated children with HCV infection progressed to decompensated cirrhosis before 16 years of age [9]. We believe that none of our subjects showed cirrhosis because of racial differences, because roughly half of them received IFN therapy with/without RBV, or because of both factors.

Relative prevalence of HCV genotypes is changing worldwide. We found genotype 1 to be decreasing, as did a previous report of children with HCV infection in Italy [11]. Genotype 2 was increasing in our Japanese survey, in contrast with increases in genotypes 3 and 4 in Italy [11]. Notably, genotype 2 has become most prevalent (57%) in our pediatric survey, although a recent report concerning adults stated relative prevalences of genotypes 1 and 2 in Japan in 2011 as 65 and 34%, respectively [4]. Toyoda et al. reported that genotype 1 remains most common in adults born before 1970, although genotype 2 has become most prevalent in adults born in or after 1970. Additionally, about half of these younger infected adults had a history of intravenous drug use or tattooing (though not of blood transfusion) [24]. These results suggest that in Japan genotype 2 may have spread to young adults by drug use or tattooing and then to children by maternal transmission. Up-to-date knowledge of genotype frequencies in Japanese children will be important in considering future treatment options against HCV infection.

Histopathology examination of liver specimens from most children with CHC showed fibrosis to be absent or mild, with inflammation predominating. No cirrhosis was found. Table 6 summarizes the largest studies of liver biopsy findings in children with CHC from Europe, the US, and Japan [14, 25, 26]. Kage et al. reported that the liver showed absent or mild fibrosis in most untreated Japanese children with CHC, as well as absence of cirrhosis. However, transmission was different in that study, with transfusion accounting for 85% of cases [14]. In the present study, even though 86% of our patients who underwent liver biopsy had maternal transmission, we observed similar histopathologic features in untreated Japanese children with CHC, including absence of fibrosis in 33% of patients and absence of cirrhosis in all. In contrast, Guido et al. reported that liver histopathology showed cirrhosis in 1% of untreated children with CHC in Italy and Spain [25], while Goodman et al. found the frequency in the US to be 2% [26]. Additionally, fibrosis was absent in smaller percentages of specimens in these studies than ours (28% [25] and 14% [26] vs. 33%). Thus, Japanese children with CHC might have less risk of fibrosis and cirrhosis than chronically infected children in some Western countries. Some reports of adults with CHC have associated patient age and duration of infection with progression of fibrosis [27, 28]. In children with CHC, the present study and Goodman et al. showed no significant correlations of degree of fibrosis with age at biopsy or duration of infection, although Guido et al. found degree of fibrosis to correlate with both patient age and duration of infection [26, 29]. Additionally, Mohan et al. reported that sequential biopsy specimens demonstrated progression of fibrosis in children with CHC, aged 8.6 ± 4.1 years at the first biopsy and 14.5 ± 4.0 years at the second [30]. Accordingly, severity of fibrosis might be more closely related to age or duration of infection in adolescence and young adulthood than in childhood.

New direct-acting antiviral agents (DAAs) now are being developed at a remarkable pace. Combining DAAs targeting different stages in the viral proliferation cycle has proven highly effective, permitting development of IFN-free and largely RBV-free regimens that might be better tolerated. Such oral regimens now have shown cure rates exceeding 90% in most adult populations [31–33]. We soon should be able to treat children with HCV infection using the new DAAs [34]. The results of our study, particularly, those concerning genotype trends and histopathologic features, should be useful to pediatric hepatologists in Japan and elsewhere in considering treatment of children with HCV infection using the new DAAs.

HCV/HIV coinfection is highly prevalent in Asia [35]. Omata et al. reported that maternal transmission of HCV is affected significantly by coinfection with HIV, and safety and efficacy of recently developed DAAs and those under development in reducing maternal transmission, particularly in the presence of HIV coinfection, require further investigation [36]. In the present study, maternal transmission accounted for 99% in the last decade. We therefore should undertake curative treatment using new DAAs in young women with HCV/HIV coinfection before pregnancy in order to prevent maternal transmission.

An important limitation of this study is the retrospective nature of data from most patients, particularly those who are older. The group born from 1986 to 1995 is smaller than groups born from 1996 to 2005 or from 2006 to 2015, probably because of loss of patient record accessibility at pediatric centers following transition to adult health care. Clinical diagnosis at last visit and prevalence of treatment clearly differ between subjects born from 1986 to 2005 and

Table 6 Liver histologic findings in large studies of children with chronic hepatitis C

Author	Year	Country	Patients	Age at biopsy years, mean ± SD	Type of exposure, %		Fibrosis, %			
					Maternal	Transfusion	None	Mild	Bridging	Cirrhosis
Kage et al. [14]	1997	Japan	109	8.8 ± 4.2	11	85	96[a]		4	0
Guido et al. [25]	1998	Italy/ Spain	80	9.1 ± 4.8	60	24	28	55	16	1
Goodman et al. [26]	2008	US	121	9.8 ± 3.7	78	7	14	80	4	2
Present study	2017	Japan	147	8.9 ± 4.0	86	7	33	58	9	0

Fibrosis staging as follows: none, F0 or Ishak 0; mild, F1 or Ishak 1–2; bridging, F2-3 or Ishak 3–4; cirrhosis, F4 or Ishak 5–6

SD standard deviation

[a] Total of none and mild

those born from 2006 to 2015 because of differing length of the follow-up period.

In conclusion, we clarified the epidemiologic features and natural history of Japanese children with HCV infection over the last three decades. To our knowledge, this is the largest nationwide cohort study from Asia. Age at initial diagnosis of infection has decreased significantly. Cirrhosis and hepatocellular carcinoma did not develop. The proportion of maternal transmission significantly increased in the last decade to 99%. No transfusion-related cases have been seen since 1994. Genotype 2 has become most prevalent among Japanese children. Histopathologic examination of the liver showed fibrosis to be absent or mild in most children with CHC.

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Authors' contributions TM, TT, and HT contributed to the concept and design of the study. All authors contributed to analysis and interpretation of the data. TM and HT contributed to writing the manuscript. Thus, all authors contributed to the manuscript.

Compliance with ethical standards

Conflict of interest We have no conflict of interest.

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Short Communication

Suppression of hepatitis B surface antigen production by combination therapy with nucleotide analogues and interferon in children with genotype C hepatitis B virus infection

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Aim: Sustained suppression of hepatitis B surface antigen (HBsAg) production after interferon (IFN) treatment has not been reported for children with genotype C chronic hepatitis B virus (HBV) infection, which is prevalent in Asia. Among children with hepatitis B envelope antigen-positive genotype C chronic HBV infection, we compared the efficacy of combination therapy with nucleotide analogues and IFN-α in 11 children with 12 historical cases treated with IFN monotherapy.

Methods: The combination of lamivudine and conventional IFN-α was introduced for the first three patients; the other eight patients were treated with entecavir and pegylated IFN.

Results: Demographic factors as well as baseline HBsAg titers and HBV-DNA levels were similar between the two groups. In the combination therapy group, viral loads were suppressed in 9/11 to below 4.0 log copies/mL both at the end of the therapy (EOT) and at 6 months after EOT. In contrast, in the IFN monotherapy group, suppression of viral loads was observed in 2/12 and 3/12 at EOT and at 6 months after EOT, respectively. In the combination therapy group, HBsAg titers dropped from 4.03 at pretreatment to 2.91 log IU/mL at 6 months after EOT with 4/11 showing a drop to below 1000 IU/mL (one patient achieved HBsAg clearance). In contrast, the amount of HBsAg did not change during the corresponding periods in the IFN monotherapy group.

Conclusions: Our preliminary results suggest that combination therapy might be effective in the suppression of HBsAg production as well as HBV-DNA production for children with genotype C chronic HBV infection.

Key words: genotype C, HBeAg seroconversion, HBsAg seroconversion, interferon, nucleotide analogue

INTRODUCTION

INTERFERON (IFN) IS a standard therapy of care for children with chronic hepatitis B virus (HBV) infection.[1] However, IFN monotherapy has not been satisfactory in promoting hepatitis B surface antigen (HBsAg) clearance in children or adults in Japan.[2] Moreover, sustained suppression of HBsAg production after IFN treatment was not reported for children with chronic hepatitis B, including genotype C chronic HBV infection, which is prevalent in Asia.

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In adult patients, HBsAg loss after tenofovir plus pegylated interferon-α (PEG-IFN) therapy was recently reported and suppression of HBsAg production by combination therapy was associated with HBV genotype A.[3] Our survey of published work failed to find any reports on the efficacy of this combination therapy in children with genotype C chronic HBV infection. In this study, we investigated the efficacy of combination therapy with nucleotide analogues and IFN-α in terms of suppression of HBsAg production as well as other biochemical and virological responses, including alanine aminotransaminase (ALT) normalization, hepatitis B envelope antigen (HBeAg) seroconversion, and suppression of HBV-DNA levels.

METHODS

FROM 2010 TO 2016, 39 patients with HBeAg-positive genotype C chronic HBV infection and their guardians

visited our center. Twenty-one of the 39 patients who had a sustained elevation in ALT for more than 6 months had the therapy explained to them. Eleven of the 21 agreed to enroll in the trial therapy (combination therapy group) whereas the other 10 patients had therapy withheld. The remaining 18 had never experienced an elevation in ALT levels and were regarded as asymptomatic carriers. An elevation in ALT levels was defined as a level >60 IU/L according to Jonas *et al.*[1]

As a comparison, registered cases that had received IFN monotherapy or PEG-IFN monotherapy were searched using the medical records of children with chronic HBV infection, which were collected in a nation-wide survey.[4] We identified 82 patients with IFN monotherapy and 14 patients with PEG-IFN monotherapy. Among them, 12 patients with IFN monotherapy and four patients with PEG-IFN monotherapy met the following inclusion criteria: pretreatment HBeAg positivity, availability of laboratory data including ALT, HBsAg, HBeAg, and HBV-DNA both at baseline and at 6 months after the end of therapy (EOT), and completion of the scheduled treatment regimen as described below. On evaluation of an efficacy of combination therapy, only cases with IFN monotherapy were compared because the number of eligible cases with PEG-IFN monotherapy was too small to compare with the combination therapy group.

The effect on HBsAg production as well as circulating levels of ALT, HBeAg, and HBV-DNA were assessed prior to therapy, at EOT, and every 6 months after EOT in the 11 children with genotype C chronic HBV infection. Liver biopsy specimens were evaluated for the activity of hepatitis and the degree of fibrosis according to the classification of Desmet *et al.*[5]

Treatment regimen

Combination therapy consisted of nucleotide analogues for the first 3 months using lamivudine 3 mg/kg/day plus natural IFN-α 0.1 MU/kg body weight three times a week for 6 months in the first three patients, or entecavir 0.01 mg/kg/day plus PEG-IFN 180 μg/m^2 body surface area weekly for 6 months in the remaining eight patients. The IFN monotherapy group received natural IFN-α 0.1 MU/kg body weight three times a week for 24 weeks. The PEG-IFN monotherapy group received 180 μg/m^2 body surface area weekly for 48 weeks.

Statistical analysis

Differences in mean values and the frequency of patients' characteristics between groups were compared using the Mann–Whitney *U*-test and the Fisher's exact test, respectively. All statistical analyses were based on two-sided hypotheses tested with a significance level of $P < 0.05$.

Ethical considerations

The study protocol complied with the ethical guidelines of the Declaration of Helsinki of 1975 (2004 revision) and was approved by the Ethics Committee of Osaka General Medical Center (Osaka, Japan).

RESULTS

Demographic data of children with HBeAg-positive genotype C chronic HBV infection

THE 11 CHILDREN who underwent the combination therapy from 2010 to 2016 consisted of seven boys and four girls with the average age of 9.2 years at treatment (Table 1). Transmission routes were mother to child in nine patients, father to child in one patient, and grandfather to child in one. Baseline factors including age at treatment, gender, transmission routes, and duration of observation were similar between the two groups. Baseline ALT values were greater in the combination therapy group than in the IFN monotherapy group, although it did not reach statistical significance. Both baseline HBsAg titers and HBV-DNA levels were in a similar range when comparing the two groups. A liver biopsy showed a mild activity of hepatitis (A1) for all patients expect one with a

Table 1 Comparison of demographic factors among children with genotype C hepatitis B virus (HBV) infection treated with interferon (IFN) monotherapy or combination therapy

	IFN monotherapy ($n = 12$)	Combination therapy ($n = 11$)	*P*-value
Age, years†	9.2 ± 4.2	9.2 ± 2.9	NS
Male sex, *n* (%)	4 (33)	7 (62)	0.22
MTCT, *n* (%)	8 (66)	9 (81)	NS
Observation, years†	4.0 ± 1.7	3.4 ± 2.1	0.45
Baseline ALT, IU/L†	155 ± 91	440 ± 375	0.06
Peak ALT, IU/L†	450 ± 605	664 ± 346	0.41
HBsAg, log IU/mL†	4.00 ± 0.30	4.23 ± 0.24	0.11
HBV-DNA, log copies/mL			
≥9	4	4	NS
8.0–8.9	4	5	
7.0–7.9	4	2	

†Mean ± standard deviation.
ALT, alanine aminotransaminase; IFN, interferon; MTCT, mother-to-child transmission; NS, not significant.

moderate degree of hepatitis (A2) (data not shown). A moderate degree of fibrosis (F2) was noted in all patients.

Natural course of children who had combination therapy withheld

Ten patients were followed for ALT, HBsAg, HBeAg, and HBV-DNA with no treatment for a median of 2.7 years. One of the 10 has had spontaneous seroconversion to HBeAb positive/HBeAg negative after 16 months of follow-up. In the remaining nine patients, HBeAg has remained positive.

Outcome of children with combination therapy or IFN monotherapy

In the combination therapy group, titers of HBeAg were rapidly decreased during the 6 months of therapy in all patients and suppressed in the negative range in eight of the 11 at EOT. Thereafter a loss of HBeAg occurred in two patients and remained positive in one patient at 6 months after EOT (Fig. 1). Hepatitis B envelope antigen seroconversion was significantly higher in the combination therapy group than in the untreated group (90.9% vs. 10.0%, $P \leq 0.001$). The seroconversion rate at 6 months after EOT was also greater in the combination therapy group than in the IFN monotherapy group ($P = 0.027$; Table 2a).

Viral loads were decreased in all patients of the combination therapy group during therapy and were suppressed in most of the patients to below 4.0 log copies/mL (LC/mL) both at EOT and at 6 months after EOT (Fig. 2a). In contrast, in the 12 patients of the IFN monotherapy group, the same degree of suppression of viral loads during the corresponding observation period was observed in only two and three patients at EOT and at 6 months after EOT, respectively (Fig. 2b). The decrease in viral loads at 6 months after EOT was more frequently seen in the combination therapy group than in the IFN monotherapy group ($P = 0.012$; Table 2a).

In the combination therapy group, HBsAg titers substantially dropped from 4.03 at pretreatment to 2.91 log IU/mL at 6 months after EOT: five of the 11 patients showed more than a 1.0-log drop in the HBsAg titers and in four of the five patients it decreased to <1000 IU/mL (Fig. 3a). Of note, one of the five patients achieved HBsAg clearance at 12 months after EOT (case 3). In contrast, the HBsAg levels did not change during the corresponding observation period in the IFN monotherapy group (Fig. 3b). The difference between the two

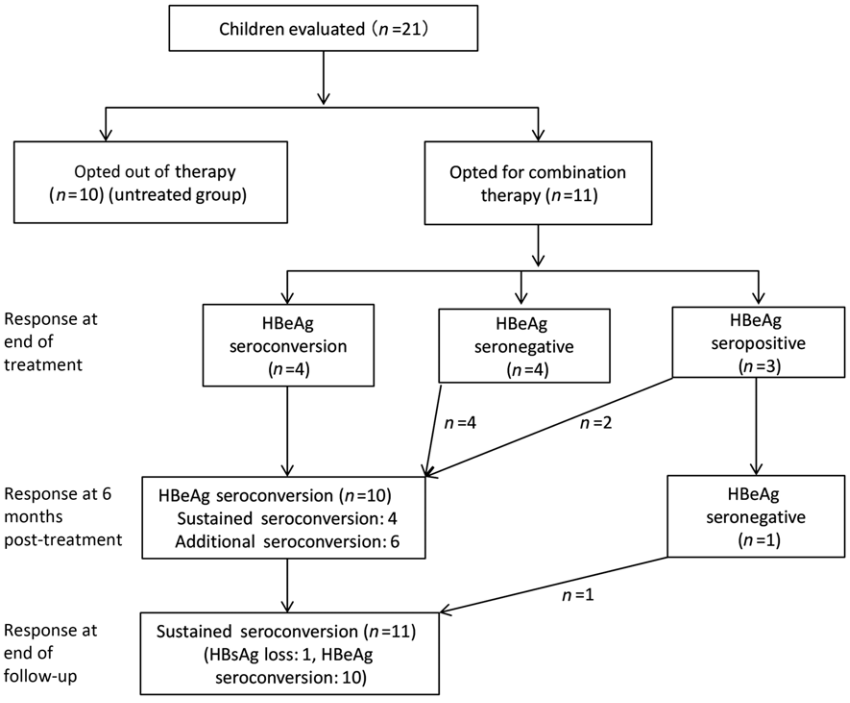

Figure 1 Flow diagram of the study of the efficacy of combination therapy with nucleotide analogues and interferon in children with genotype C hepatitis B virus infection, including summary of results. HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen.

Table 2a Comparison of efficacy between interferon (IFN) monotherapy and combination therapy groups among children with genotype C hepatitis B virus (HBV) infection

	Lamivudine plus interferon ($n = 3$)	Entecavir plus PEG-IFN ($n = 8$)	Combination therapy ($n = 11$)*	IFN monotherapy ($n = 12$)*	*P*-value*
ALT normalization	3/3	7/8	10/11	6/12	0.069
HBeAg/HBeAb seroconversion	3/3	7/8	10/11	5/12	0.027
HBV-DNA <4.0 log copy/mL	3/3	6/8	9/11	3/12	0.012
HBsAg 1.0-log drop	2/3	3/8	5/11	0/12	0.014
HBsAg <1000 IU/mL	1/3	3/8	4/11	0/12	0.037
HBsAg loss	1/3	0/8	1/11	0/12	NS

**P*-values are shown for these two groups.
ALT, alanine aminotransaminase; HBeAb, hepatitis B envelope antibody; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; NS, not significant; PEG-IFN, pegylated IFN.

Table 2b Comparison of side-effects between interferon (IFN) monotherapy and combination therapy among children with genotype C hepatitis B virus infection

	IFN monotherapy ($n = 12$)	Combination therapy ($n = 11$)	*P*-value
Leukopenia	2	1	NS
Anemia (Hb <10 g/dL)	0	0	NS
Thrombocytopenia (plt <100 000/μL)	1	1	NS
Elevated serum transaminase levels	2	1	NS
Hypothyroidism	0	0	NS
Lethargy	1	0	NS
Mental depression	0	0	NS
Hair loss	0	0	NS
Skin rash	0	0	NS

Hb, hemoglobin; NS, not significant; plt, platelets.

groups at 6 months after EOT was greater in the combination therapy group than in the IFN monotherapy group both for 1.0-log drop and for a drop below 1000 IU/mL ($P = 0.014$ and $P = 0.037$, respectively; Table 2a).

There were no differences between the first three patients treated with lamivudine plus interferon and the later eight patients with entecavir plus PEG-IFN in terms of seroconversion rate, suppression of viral loads, 1.0-log drop in HBsAg, or drop below 1000 IU/mL at 6 months after EOT (Table 2a).

Sustainability of the suppression of HBsAg production was partly shown by an 84-month follow-up in cases 2 and 3, both of which showed more than 1.0-log drop at 6 months after the end of the combination therapy (Fig. S1). Moreover, HBsAg titers decreased below 1000 IU/mL after 6 years in case 2. In the IFN monotherapy group, titers of HBsAg were available for most patients between 12 and 36 months after EOT and showed no change compared to those at 6 months after EOT (data not shown).

Outcome of children treated with PEG-IFN monotherapy

In the four patients who underwent PEG-IFN monotherapy, ALT normalization was reported in three, HBeAg seroconversion in two, and suppression of HBV-DNA in two at 6 months after EOT. The amount of HBsAg was repeatedly assessed in three of the four patients and no apparent decrement in HBsAg titers was observed in those three patients, either at EOT or 6 months after EOT.

Safety of combination therapy

A similar frequency of bone marrow suppression associated with IFN treatment was observed in the two groups; leukopenia in two and thrombocytopenia in one for the IFN monotherapy group, and one each for the combination therapy group (Table 2b). Transient elevation in serum transaminase levels was also infrequently seen in

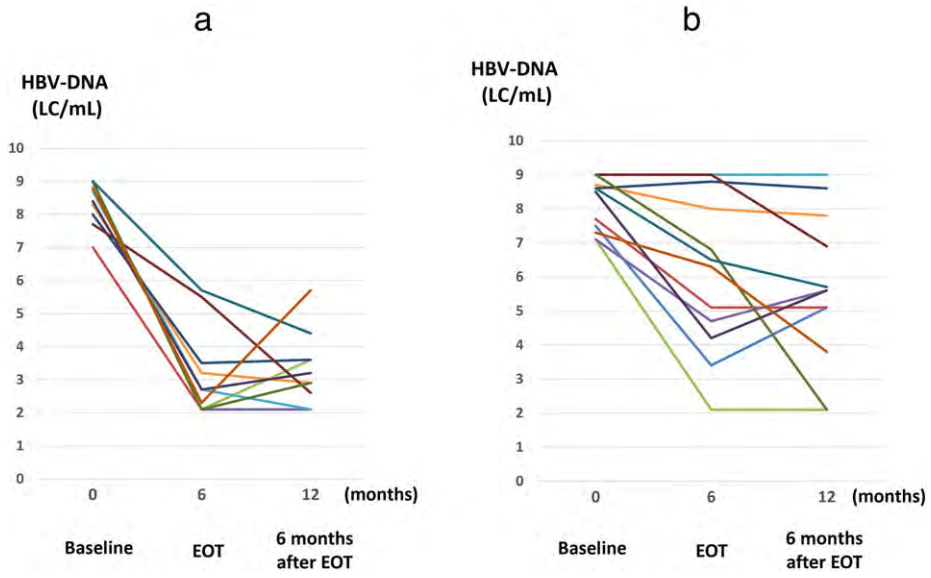

Figure 2 Hepatitis B virus (HBV)-DNA levels in two groups of children with genotype C HBV infection treated with combination therapy or interferon (IFN) monotherapy. Baseline values of each group are presented with corresponding estimations at end of treatment (EOT) and at 6 months after EOT for the combination therapy group (a) and the IFN monotherapy group (b). LC, log copies. [Color figure can be viewed at wileyonlinelibrary.com]

both groups. None of these side-effects was serious enough to warrant cessation of therapy.

DISCUSSION

IN THIS STUDY, all the 11 treated children showed a favorable response to combination therapy with IFN and nucleotide analogues. Suppression of HBeAg production occurred and serum HBV-DNA levels dropped to <4.0 LC/mL at 6 months after EOT in most patients. The mean value of HBsAg decreased from 4.03 log at baseline to 2.91 log IU/mL at 6 months among the 11 treated patients and HBsAg dropped below 1000 IU/mL in four patients. Furthermore, one of the four patients achieved

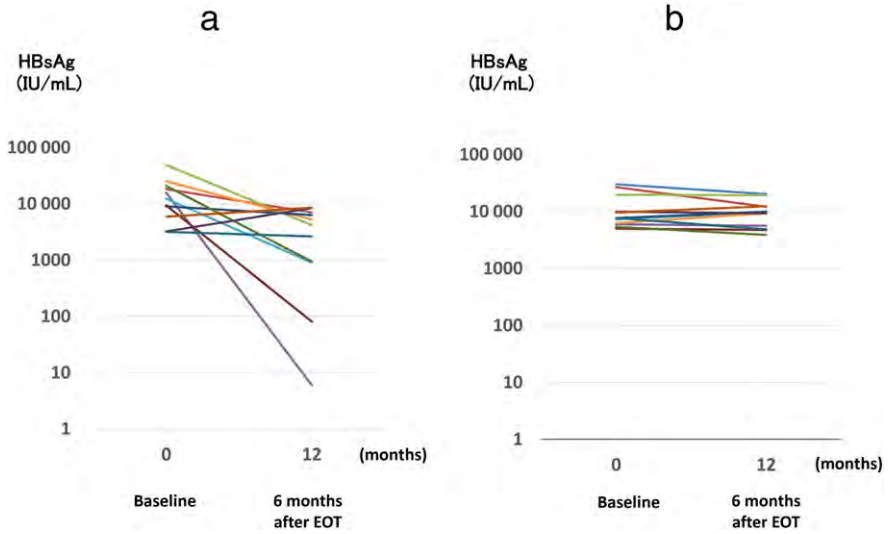

Figure 3 Hepatitis B surface antigen (HBsAg) titers (expressed as logarithms) in two groups of children with genotype C HBV infection treated with combination therapy or interferon (IFN) monotherapy. Baseline values of each group are presented with corresponding estimations at 6 months after end of treatment (EOT) for the combination therapy group (a) and the IFN monotherapy group (b). [Color figure can be viewed at wileyonlinelibrary.com]

HBsAg clearance 1 year after therapy and it was decreased below 1000 IU/mL in another patient after 6 years. The safety profile of the combination therapy group was similar to the IFN monotherapy group and no serious side-effects were observed in either group.

The first therapeutic trial in children using a similar regimen was reported by D'Antiga *et al.* in 2006.[6] They treated 23 immune-tolerant children and achieved HBeAg seroconversion in five (22%) and HBsAg loss in four (17%). All of the four patients who cleared HBsAg had genotype B HBV infection. Two of their 23 patients who had genotype C infection did not respond to the therapy. Similar combination therapy in 112 children with an ALT >1.5 times the upper limit of normal resulted in a higher response (55% vs. 27%) and more HBsAg loss (12.5% vs. 4.6%) when compared with 52 children who underwent nucleotide analogue lead-in combination therapy.[7] Twenty-eight children in an immune-tolerant phase were treated with combination therapy as reported by D'Antiga et al.[8] Eleven of the 28 become seronegative for HBeAg and five of the 11 had HBsAg clearance, but the genotype of the subjects was not examined in the latter two studies. Furthermore, these studies into the efficacy of combination therapy did not quantitatively assess the change in HBsAg production.

There have been no studies on the efficacy of combination therapy in children with genotype C chronic HBV infection. Therefore, it is unknown whether genotype C-infected children would respond to combination therapy with comparable efficacy as has been seen with genotype B in children.[6] A 20-year observation of the natural course of infection in children has shown that those with initial titers of HBsAg <1000 IU/mL were more likely to clear HBsAg than those with higher titers.[9] Accordingly, treatment-related suppression of HBsAg production <1000 IU/mL might lead to clearance of HBsAg in the near future. In this study, four of the 11 patients have achieved a suppression of HBsAg production <1000 IU/mL after the combination therapy. However, long-term observation is required to determine whether clearance of HBsAg might occur in the combination therapy group, as seen in children who showed low baseline levels of HBsAg and eventually cleared HBsAg.[9]

Our preliminary results suggest that combination therapy could be effective in suppression of HBsAg production as well as in suppression of both HBeAg and HBV-DNA production for children with chronic genotype C HBV infection. Prospective studies are needed to evaluate the efficacy of combination therapy and to clarify predictive factors of its efficacy in children with genotype C chronic HBV infection.

ACKNOWLEDGMENTS

THIS RESEARCH WAS supported by the Japan Agency for Medical Research and Development (grant no. 16fk0210310h0003).

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found online in the Supporting Information section at the end of the article.

Figure S1 Changes in hepatitis B surface antigen titers over 7 years for 11 children with genotype C hepatitis B virus infection treated with combination therapy.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Hepatitis B vaccine: Immunogenicity in an extremely low-birthweight infant

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Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan

Key words extremely low-birthweight infant, hepatitis B vaccine, hepatitis B virus, immunogenicity, mother-to-child infection.

From 2013, infants born to mothers carrying serum hepatitis B (HB) surface antigen (HBsAg) receive HB immunoglobulin at birth and HB vaccine at birth, and at 1 and 6 months of age in Japan (prevention protocol for mother-to-child HB virus infection).[1] Due to immature immune response to HB vaccine, the American Academy of Pediatrics and Japan Pediatric Society recommend that infants <2,000 g birthweight are given an additional HB vaccination at 2 months of age.[2,3] No previous case report, however, has described the trajectory of the immunogenic response for this prevention protocol, including an additional dose at 2 months of age, in extremely low-birthweight (ELBW) infants. The present case is reported with informed consent.

The present patient was born to a 29-year-old Chinese mother (gravida 0, para 0) with HBsAg. At 20 weeks of gestational age, serum HBsAg, HB envelope antigen, HB virus core-related antigen, and HB virus DNA were positive (67 878 IU/mL, 1,531.9 sample relative light units/cut-off, >7.0 log U/mL, and 9.7 log copies/mL, respectively). Both serum HB surface antibody (HBsAb) and HB envelope antibody were negative. The HB virus genotype was type C. A male newborn weighing 918 g was born at 25 weeks and 4 days of gestational age via cesarean section due to fetal distress.

He was admitted to the neonatal intensive care unit due to ELBW. Along with respiratory and circulatory treatment, i.v. immunoglobulin (IVIG; 500 mg/10 mL, Venoglobulin IH™, Japan Blood Products Organization, Tokyo, Japan) was administered soon after birth because of hypoimmune globulinemia (serum total IgG, 280 mg/dL). At 11 h after birth, a total of 200 U/mL HB immune globulin (Dried HB globulin Nichiyaku™; Nihon Pharmaceutical, Tokyo, Japan) was injected i.m. in the right and left femoral muscles (100 U/0.5 mL in each side), and HB vaccine (0.25 mL, Bimmugen™; Kaketsuken, Kumamoto, Japan) was injected s.c. in the left upper arm. No side-effects, such as redness, swelling, or induration were observed. HB vaccine was again administered at 1 and at 2 months of age. The infant was reared on breast milk and was discharged at 4 months of age. The fourth HB vaccine was injected at 6 months of age.

The HBsAb titer reached a peak at 1 month of age, and decreased to the lowest level at 4 months of age, but HBsAb was >10 mIU/mL (Fig. 1). Then, the HBsAb titer gradually increased, and after the fourth HB vaccine, it finally increased to >100 mIU/mL at 12 months of age. Serum HBsAg was negative at 12 months of age.

We herein report the HBsAb titer in an ELBW infant who received four doses of HB vaccine. In the present case, the prevention protocol for mother-to-child HB virus infection with an additional dose at 2 months of age (0, 1, 2, and 6 months of age) achieved sufficient seropositivity of HBsAb at 12 months of age. The infant had an HBsAb titer of 47 mIU/mL at the time of discharge, even with an additional vaccine at 2 months of age. Because ELBW infants are usually discharged from hospital at 3–4 months of age, and are

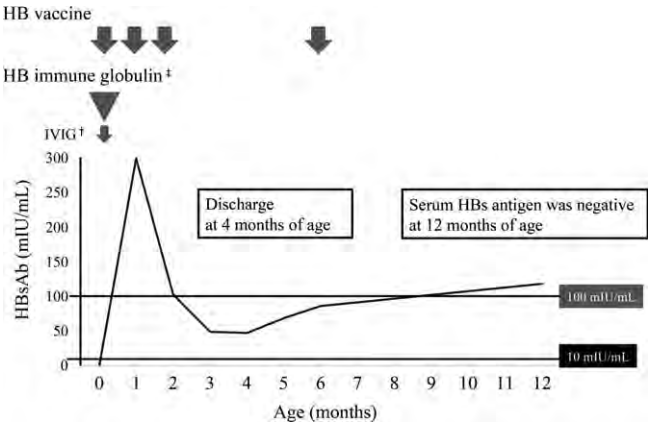

Fig. 1 Trajectory of serum hepatitis B surface antibody (HBsAb) titer. †Effect of i.v. immunoglobulin (IVIG) on HBsAb titer: the patient received 500 mg/10 mL Venoglobulin IH™ (Japan Blood Products Organization), which has an HBsAb titer of approximately 100 mIU/mL. Assuming that the circulating blood volume is 72 mL (80 mL/kg bodyweight) and the bioavailability of IVIG is 100%, IVIG treatment might have increased HBsAb titer by 14 mIU/mL. Given, however, that the half-life of Ig is 27 days,[4] the effect is limited. ‡Effect of HB immune globulin on HBsAb titer: the titer at 4 months of age (47 mIU/mL) can be explained only by the HB immune globulin at birth because the half-life of HB immune globulin is 23 days.[5]

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doi: 10.1111/ped.13547

then in close contact with their mother who are HB virus carriers, it is important for the ELBW infant to have a sufficient HBsAb titer at that time.

The seroprotection level is usually defined as HBsAb titer ≥10 mIU/mL.[6,7] Although all infants ≥2,000 g birthweight who received three doses of HB vaccine at 0, 1, and 6 months of age at the present hospital had sufficient HBsAb (median, 210 mIU/mL; range, 21–898 mIU/mL; n = 12), in a previous study, ELBW infants who received three doses of HB vaccinations at birth and at 1–3 and at 6–8 months of age had only a 52% seropositivity rate.[6] And in another study, 98.4% of preterm infants vaccinated using another four-dose HB vaccine protocol (0, 1, 2, and 12 months of age) had a protective level.[7] Four doses of HB vaccine may be needed to obtain a sufficient rate of seropositivity in ELBW infants as recommended by the Japan Pediatric Society.

Acknowledgments

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Disclosure

Outside the submitted work, I.M. has received grants from Japan Blood Product Organization, Daiichi Sankyo Co., Ltd., MSD Co., Ltd., AbbVie LLC, Taisho Toyama Pharmaceutical Co., Ltd., and Air Water Inc.; lecture fees from MSD Co., Ltd., Pfizer Japan, Inc., Novo Nordisk Pharma Ltd., Shionogi Co., Ltd., AbbVie LLC, Japan Vaccine Co., Ltd., Asahikasei Medical Co., Ltd., and Atom Medical Corp.; manuscript fees from Atom Medical Corp., Sanofi K.K., Asahikasei Medical Co., Ltd., and Japan Blood Product Organization,; and honoraria from Sanofi K.K. K.I. has received grants from Novartis Pharma K.K., Japan Blood Product Organization, Pfizer Japan, Inc., Kyowa Hakko Kirin Co., Ltd., AbbVie LLC, JCR Pharmaceuticals Co., Ltd., Daiichi Sankyo, Co., Ltd., Genzyme Japan K.K., Teijin Pharma Ltd., Miyarisan Pharmaceutical Co., Ltd., CSL Behring, Novo Nordisk Pharma Ltd., Air Water Inc., and Astellas Pharma Inc., Lecture fees from Pfizer Japan, Inc., Asahi Kasei Pharma Corp., Kowa Pharmaceutical Co., Ltd., MSD Co., Ltd., Alexion Pharmaceuticals, AstraZeneca K.K., Meiji Seika Pharma Co., Ltd., Novartis Pharma K.K., Zenyaku Kogyo Co., Ltd., Daiichi Sankyo, Co., Ltd., Springer Japan, Medical Review Co. Ltd., Chugai Pharmaceutical Co., Ltd., Boehringer Ingelheim, and Nikkei Radio Broadcasting Corporation, manuscript fees from Chugai Pharmaceutical Co., Ltd., and consulting fees from Zenyaku Kogyo Co., Ltd., Astellas Pharma Inc., Ono Pharmaceutical Co., Ltd. and Takeda Pharmaceutical Co., Ltd. The other authors declare no conflict of interest.

Author contributions

K.Y. and I.M. drafted the initial manuscript. K.Y. and S.I. collected the clinical data. K.Y., I.M. and K.F. interpreted the data. K.I. revised the article critically for important intellectual content. All authors contributed to the intellectual content of this manuscript and approved the final manuscript as submitted.

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厚生労働行政推進調査事業費（肝炎等克服緊急対策研究事業）

肝炎ウイルスの新たな感染防止
－残された課題・今後の対策－

平成 30 年度 総括・分担研究報告書

発行：平成 31(2019) 年 3 月

研究代表者 四柳　宏

東京大学医科学研究所先端医療研究センター 感染症分野

東京大学医科学研究所倫理審査委員会
審査結果通知書

平成３０年１２月２７日

申請者
感染症分野
四柳　宏　教授　殿

東京大学医科学研究所長
村上　善則

審査番号：　３０－６１
承認番号：　３０－６１－Ｂ１２２７
研究課題：　医療従事者へのB型肝炎ワクチン接種状況に関するアンケート調査
申請日：　平成３０年１２月２７日
審査委員会名：　倫理審査委員会第二委員会

上記研究計画について、平成３０年１２月２０日開催の本委員会における指摘事項の修正を確認し、下記のとおり決定しましたので、ここに通知します。

記

判定	
[承認]	~~変更の勧告~~
~~条件付き承認~~	~~否承認~~
□修正を要する	~~非該当~~
□修正不要	
理由・コメント	

整理番号	CRB-18-03-002
区分	□特定臨床研究■非特定臨床研究
	□医薬品□医療機器□再生医療等

2018年11月7日

臨床研究実施許可通知書

小児科・新生児科
髙野　智子　　様

2018年11月7日付け審査結果通知書にて承認された臨床研究について、実施を許可致します。

記

臨床研究課題名	保育の場における肝炎ウイルス感染予防の理解及び実践を図るための保育施設勤務者に対するアンケート調査

以上

大阪急性期・総合医療センター
総長

別記様式４

臨床研究倫理審査結果通知書

平成３０年１２月２８日

申請者（実施責任者）
岩淵　敦　殿

筑波大学附属病院長　　原　晃

平成３０年９月１３日付けで倫理審査申請のありました臨床研究の実施について、審査の結果、下記のとおり判定しましたので通知します。

記

１　臨床研究題目（H30-220）
「Ｂ型肝炎ワクチン定期接種化後の本邦小児におけるＢ型肝炎ウイルス感染およびワクチン接種の実態調査」

２　判　定
■　承　認
□　条件付承認
□　変更の勧告
□　不承認
□　非該当

３　理由等（判定が承認以外の場合）

研究期間　2018 年 12 月 28 日～2022 年 3 月 31 日
（ただし、臨床研究保険に加入する場合の研究開始日は、臨床研究保険補償開始日とする。）

整理番号

RK-190130-1

書式６　Ver. 1610

西暦２０１９年２月１３日

臨床研究　審査結果通知書

日本大学医学部附属板橋病院　病院長殿

日本大学医学部附属板橋病院
臨床研究倫理審査委員会
東京都板橋区大谷口上町３０番１号
委員長　武井　正美　　印

審査依頼のあった件について審査結果を下記のとおり報告いたします。

記

研究課題名	Ｂ型肝炎ワクチン定期接種化後の本邦小児におけるＢ型肝炎ウイルス感染及びワクチン接種の実態調査
審査事項 （審査資料）	■研究の実施の適否 ■臨床研究　申請書（西暦 2019年1月11日付） □臨床研究実施医療機関の概要書（西暦　年　月　日作成） □研究の継続の適否 □臨床研究　実施状況報告書（西暦　年　月　日付） □臨床研究　変更申請書（西暦　年　月　日付） □臨床研究における重篤な有害事象に関する報告書 （西暦　年　月　日付） □その他（　　）
研究期間	承認日　～　2022年3月31日
審査区分	■委員会審査（審　査　日：　2019年　2月 12日） □迅速審査　（審査終了日：　年　月　日）
審査結果	□承認　■条件付承認　□却下　□既承認事項の取り消し　□保留
指摘事項および理由・条件等	別紙(1902-07)のとおり
備考	別紙＜注意事項＞のとおり

西暦２０１９年 3 月 / 日

申請者(研究責任者)

小児・新生児病科

新生児病科外来医長　岡橋　彩　殿

申請のあった研究に関する審査事項について上記のとおり決定しましたので通知いたします。

日本大学医学部附属板橋病院　病院長　徳橋　泰明

2019 年 3 月 / 日 条件が満たされたことを確認しました。

日本大学医学部附属板橋病院　病院長

神小医第62号
平成31年3月25日

神戸大学大学院医学研究科内科系講座
小児科学分野こども急性疾患学部門
野津寛大　様

神戸こども初期急病セ
センター長　石田

神戸こども初期急病センター倫理委員会審査結果について(通知)

平成31年1月21日付けで倫理審査申請のありました「B型肝炎ワクチン定期接種化後の本邦小児におけるB型肝炎ウイルス感染およびワクチン接種の実態調査」について、倫理委員会委員長より、承認する旨の答申がありましたので通知いたします。

記

1. 答申日　平成31年3月25日

2. 参考資料　・答申書(写)

3. その他　当該研究に係る研究計画と経過、更に結果(成果)について継続的にセンターに報告し、寄附講座ホームページに掲載する等、広報に留意ください。

以上

様式２

国立感染症研究所ヒトを対象とする医学研究倫理審査結果通知書

平成３０年９月２５日

相崎　英樹　殿

国立感染症研究所長

受付番号：９２７

研究課題名：HIV感染同性愛者における急性Ａ型、Ｃ型肝炎の解析

研究者名：相崎　英樹・井戸田　一朗・三田　英治・遠藤　知之・四柳　宏・鈴木　亮介・清原　知子・杉山　隆一・村松　正道

研究期間：２０１８年承認日～２０２２年３月末日

上記課題名の研究計画・公表予定は、国立感染症研究所ヒトを対象とする医学研究倫理審査委員会において審議され、下記のとおり判定したので通知します。

記

判定	非該当	承認	条件付承認
	変更の勧告	不承認	
勧告あるいは条件・理由			

平成３１年　4月 18 日

厚生労働大臣　　殿

機関名　国立大学
所属研究機関長　職　名　総長
氏　名　五神　真

次の職員の平成３０年度厚生労働行政推進調査事業費の調査研究における、倫理審査状況及び利益相反等の管理については以下のとおりです。

１．研究事業名　肝炎等克服政策研究事業

２．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

３．研究者名　（所属部局・職名）　医科学研究所　・　教授

（氏名・フリガナ）　四柳　宏　・　ヨツヤナギ　ヒロシ

４．倫理審査の状況

	該当性の有無	左記で該当がある場合のみ記入（※1）		
	有　無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□　■	□		□
遺伝子治療等臨床研究に関する指針	□　■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	■　□	■	東京大学	□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□　■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　）	□　■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

５．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

６．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

平成　３１年3　月4　日

厚生労働大臣　殿

機関名　東京大学

所属研究機関長　職　名　総長

氏　名　五神　真

次の職員の平成30年度厚生労働行政推進調査事業費補助金の調査研究における、倫理審査状況及び利益相反等の管理については以下のとおりです。

１．研究事業名　肝炎等克服政策研究事業

２．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

３．研究者名　（所属部局・職名）　医学部附属病院・特任教授

（氏名・フリガナ）　田倉　智之・タクラ　トモユキ

４．倫理審査の状況

	該当性の有無	左記で該当がある場合のみ記入（※1）		
	有　無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□　■	□		□
遺伝子治療等臨床研究に関する指針	□　■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	□　■	□		□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□　■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　　）	□　■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

５．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

６．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

厚生労働大臣　殿

平成31年4月4日

機関名　国立感染症研究所

所属研究機関長　職　名　所長

氏　名　脇田　隆[illegible]

次の職員の平成３０年度厚生労働科学研究費の調査研究における、倫理審査状況及び
いては以下のとおりです。

１．研究事業名　肝炎等克服政策研究事業

２．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

３．研究者名　（所属部局・職名）ウイルス第二部・室長

（氏名・フリガナ）相崎英樹・アイザキヒデキ

４．倫理審査の状況

	該当性の有無	左記で該当がある場合のみ記入（※1）		
	有　無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□ ■	□		□
遺伝子治療等臨床研究に関する指針	□ ■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	■ □	■	国立感染症研究所	□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□ ■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　）	□ ■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

５．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

６．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

２０１９年　４月　　５日

厚生労働大臣　殿

独立行政法人国立病院機構
機関名　　長崎医療センター

所属研究機関長　職　名　　院　長

氏　名　　江　崎　宏

次の職員の平成３０年度厚生労働科学研究費の調査研究における、倫理審査状況及び利益相
いては以下のとおりです。

１．研究事業名　肝炎等克服政策研究事業

２．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

３．研究者名　（所属部局・職名）　臨床研究センター・臨床研究センター長

（氏名・フリガナ）　八　橋　　弘・ヤツハシ　ヒロシ

４．倫理審査の状況

	該当性の有無	左記で該当がある場合のみ記入（※1）		
	有　無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□　■	□		□
遺伝子治療等臨床研究に関する指針	□　■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	□　■	□		□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□　■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　　）	□　■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

５．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

６．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

平成　31　年 4 月 24 日

厚生労働大臣　殿

機関名　東京大学

所属研究機関長　職　名　総長

氏　名　五神　真

次の職員の平成30年度厚生労働行政推進調査事業費補助金の調査研究における、倫理審査状況及び利益相反等の管理については以下のとおりです。

1．研究事業名　肝炎等克服政策研究事業

2．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

3．研究者名　（所属部局・職名）　医学部附属病院・教授

（氏名・フリガナ）　森屋　恭爾・モリヤ　キョウジ

4．倫理審査の状況

	該当性の有無		左記で該当がある場合のみ記入（※1）		
	有	無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□	■	□		□
遺伝子治療等臨床研究に関する指針	□	■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	■	□	■	東京大学医科学研究所倫理委員会第二委員会	□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□	■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　）	□	■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

5．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

6．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

平成３１年３月２９日

厚生労働大臣　殿

機関名　佐賀大学

所属研究機関長　職　名　学長

氏　名　宮﨑　耕治

次の職員の平成３０年度厚生労働行政推進調査事業費の調査研究における、倫理審査状況及び利益相反等の管理については以下のとおりです。

１．研究事業名　肝炎等克服政策研究事業

２．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

３．研究者名　（所属部局・職名）　附属病院・特任教授

（氏名・フリガナ）　江口　有一郎・エグチ　ユウイチロウ

４．倫理審査の状況

	該当性の有無	左記で該当がある場合のみ記入（※1）		
	有　無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□　■	□		□
遺伝子治療等臨床研究に関する指針	□　■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	□　■	□		□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□　■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　　）	□　■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。

（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

５．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

６．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

平成31年　3月 29 日

厚生労働大臣　　殿

機関名　公立大学法人名[redacted]

所属研究機関長　職　名　　理事長

氏　名　郡　健二郎

次の職員の平成30年度厚生労働行政推進調査事業費の調査研究における、倫理審査状況及び利益相反等の管理については以下のとおりです。

1．研究事業名　肝炎等克服政策研究事業

2．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

3．研究者名　（所属部局・職名）　大学院医学研究科・教授

（氏名・フリガナ）　田中　靖人・タナカ　ヤスヒト

4．倫理審査の状況

	該当性の有無	左記で該当がある場合のみ記入（※1）		
	有　無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□　■	□		□
遺伝子治療等臨床研究に関する指針	□　■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	■　□	■	公立大学法人名古屋市立大学	□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□　■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　　）	□　■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

5．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

6．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

平成31年　3月 27 日

厚生労働大臣　　殿

機関名　公立大学法人名[redacted]

所属研究機関長　職　名　　理事長

氏　名　郡　健二郎

次の職員の平成30年度厚生労働行政推進調査事業費の調査研究における、倫理審査状況及び利益相反等の管理については以下のとおりです。

1．研究事業名　肝炎等克服政策研究事業

2．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

3．研究者名　（所属部局・職名）　大学院医学研究科・研究員

（氏名・フリガナ）　細野　覚代・ほその　さとよ

4．倫理審査の状況

	該当性の有無		左記で該当がある場合のみ記入（※1）		
	有	無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□	■	□		□
遺伝子治療等臨床研究に関する指針	□	■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	■	□	■	公立大学法人名古屋市立大学	□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□	■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　　）	□	■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

5．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

6．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

2019年　4月　10日

厚生労働大臣
（国立医薬品食品衛生研究所長）　殿
（国立保健医療科学院長）

機関名　日本大学医

所属研究機関長　職　名　医学部長

氏　名　高山　忠利

次の職員の平成　　年度厚生労働科学研究費の調査研究における、倫理審査状況及び利益相反等の管理については以下のとおりです。

１．研究事業名　肝炎等克服政策研究事業

２．研究課題名　肝炎ウィルスの新たな感染防止・残された課題・今後の対策

３．研究者名　（所属部局・職名）　小児科学系小児科学分野・教授

（氏名・フリガナ）　森岡　一朗　（モリオカ　イチロウ）

４．倫理審査の状況

	該当性の有無	左記で該当がある場合のみ記入（※1）		
	有　無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□　■	□		□
遺伝子治療等臨床研究に関する指針	□　■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	■　□	■	日本大学板橋病院	□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□　■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　　）	□　■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

５．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

６．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

2019年　3月　4日

厚生労働大臣　　殿

機関名　地方独立行政法人大阪府立病院機構
大阪急性期

所属研究機関長　職　名　総長

氏　名　後藤　満-

次の職員の平成30年度厚生労働行政推進調査事業費の調査研究における、倫理審査状況及び利益相反等の管理については以下のとおりです。

1．研究事業名　肝炎等克服政策研究事業

2．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

3．研究者名　（所属部局・職名）　小児科・部長

（氏名・フリガナ）　高野　智子（タカノ　トモコ）

4．倫理審査の状況

	該当性の有無		左記で該当がある場合のみ記入（※1）		
	有	無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□	■	□		□
遺伝子治療等臨床研究に関する指針	□	■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	■	□	■	臨床研究審査委員会	□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□	■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　　）	□	■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
（※3）廃止前の「疫学研究に関する倫理指針」や「臨床研究に関する倫理指針」に準拠する場合は、当該項目に記入すること。

5．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

6．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。

31年3月28日

厚生労働大臣　　殿

機関名　茨城県立こど[redacted]

所属研究機関長　職　名　病院長

氏　名　須磨崎　亮

次の職員の平成30年度厚生労働行政推進調査事業費の調査研究における、倫理審査状況及び利益相反等の管理については以下のとおりです。

1．研究事業名　肝炎等克服政策研究事業

2．研究課題名　肝炎ウイルスの新たな感染防止・残された課題・今後の対策

3．研究者名　（所属部局・職名）　小児医療・がん研究センター　研究員

（氏名・フリガナ）　酒井　愛子　・　サカイ　アイコ

4．倫理審査の状況

	該当性の有無	左記で該当がある場合のみ記入（※1）		
	有　無	審査済み	審査した機関	未審査（※2）
ヒトゲノム・遺伝子解析研究に関する倫理指針	□　■	□		□
遺伝子治療等臨床研究に関する指針	□　■	□		□
人を対象とする医学系研究に関する倫理指針（※3）	■　□	■	筑波大学附属病院	□
厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針	□　■	□		□
その他、該当する倫理指針があれば記入すること（指針の名称：　　）	□　■	□		□

（※1）当該研究者が当該研究を実施するに当たり遵守すべき倫理指針に関する倫理委員会の審査が済んでいる場合は、「審査済み」にチェックし一部若しくは全部の審査が完了していない場合は、「未審査」にチェックすること。

その他（特記事項）

（※2）未審査に場合は、その理由を記載すること。
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5．厚生労働分野の研究活動における不正行為への対応について

研究倫理教育の受講状況	受講 ■　未受講 □

6．利益相反の管理

当研究機関におけるＣＯＩの管理に関する規定の策定	有 ■　無 □（無の場合はその理由：　　）
当研究機関におけるＣＯＩ委員会設置の有無	有 ■　無 □（無の場合は委託先機関：　　）
当研究に係るＣＯＩについての報告・審査の有無	有 ■　無 □（無の場合はその理由：　　）
当研究に係るＣＯＩについての指導・管理の有無	有 □　無 ■（有の場合はその内容：　　）

（留意事項）　・該当する□にチェックを入れること。
・分担研究者の所属する機関の長も作成すること。