Fig. 2. 成因別にみた肝細胞癌死亡の推移（推計値）
［人口動態統計および全国原発性肝癌追跡調査報告の資料を元に推計（J. Tanaka）］

されHCV感染の診断が可能となったことから，増加していた肝癌死亡のほとんどがHCVの持続感染に起因するものであることが明らかとなった．一方，2000年以後非B非C型に由来する肝癌の割合が全体の10〜15%を占め，徐々に増加傾向にあり，その原因や動向について非アルコール性脂肪性肝炎（non-alcoholic steatohepatitis：NASH）との関連性が示唆されている．

　以上より，わが国の肝細胞癌死亡の約8〜9割は肝炎ウイルス感染，HBVあるいはHCVの持続感染に起因すると考えられると同時に，そのうちの8割，すなわち肝細胞癌死亡全体の約7割はHCVの持続感染に起因するものであり，肝癌対策を構築するうえでも，HCV持続感染者（HCVキャリア）の規模の把握や治療を含むキャリア対策，感染予防対策が効果的であると考えられる．

肝癌死亡の地理的分布と肝炎ウイルスキャリア率○

　肝癌死亡の地域別分布について検討するために，全国市町村別の肝癌標準化死亡比（SMR；Bayes推定量分布図）を1971年から5年刻みに2005年まで7期別に算出した．そのうち全国の

2つの期，広島県と大阪府の3つの期について Fig. 3に示す．肝癌標準化死亡比は全国平均を100として市町村別にその高低が示されるが，1971〜1975年（第1期）では肝癌死亡の地理的に顕著な地域差は認められない．一方，2001〜2005年（第7期）では，西日本地域を中心に標準化死亡比の高い地域が認められる．広島県と大阪府における市町村別にみた肝癌死亡比の地図を例として示すと，前者は県東部沿岸を中心に2000年代に入り依然として標準化死亡比の経年的増加が認められる一方，後者は1990年代にピークを迎え減少傾向にあると考えられる．このようにわが国では，地域と時期により異なった肝癌標準化死亡比の変遷が観察できる．

　肝癌死亡の主な病因は肝炎ウイルスの持続感染に起因することが明らかとなったが，わが国の年代別にみた肝炎ウイルス感染の状況を知るために，2000年以後に得られた2つの大規模集団の特性を考慮したうえで算出した年齢階級別の肝炎ウイルスキャリア率を全国8地域に分けて Fig. 4に示す．2つの大規模集団のうち，一つは日本赤十字血液センターにおける2001〜2006年の6年
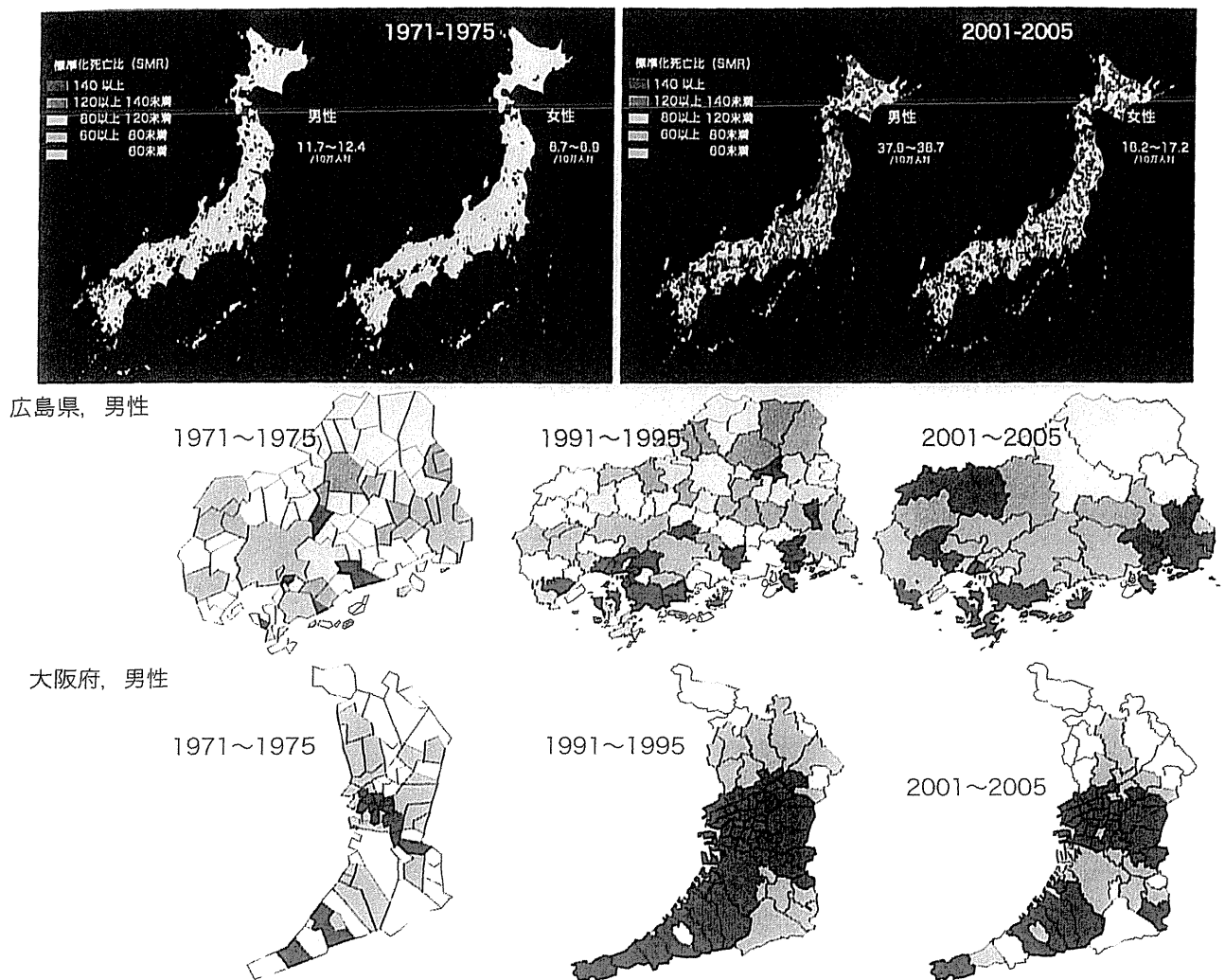
Fig. 3. 市町村別にみた肝癌標準化死亡比（Bayesian method）の経年推移（1971〜2005 年）
［厚生労働省：肝炎ウイルス感染状況・長期経過と予後調査および治療導入対策に関する研究班 2010 年報告より］

間の「初回供血者」3,748,422 人の資料から，日本赤十字社の協力の元に厚生労働省疫学班研究として算出した，20〜39 歳（2005 年時点の年齢換算）の 5 歳刻みの年齢階級別 HBs 抗原陽性率および HCV 抗体陽性率の成績である．

　もう一つは，2002 年度から 5ヵ年計画で実施された「肝炎ウイルス検診」の成績[3]のうち，「節目検診」（40〜70 歳の 5 歳刻みの節目の年齢にあたる人を対象とした検診）から得た年齢階級別 HBs 抗原陽性率および HCV キャリア率である．

　HBs 抗原陽性率（HBV キャリア率）をみると，8 地域ともに団塊の世代と考えられる 2005 年時点の年齢換算で 60 歳前後の年齢層で緩やかな一峰

性を示し，北海道九州地域で全国平均（60 歳前後，1.4%）よりも高い値が認められる．20 歳以下の若い集団ではいずれの地域も 0.1% 以下の低い値を示している．一方，HCV キャリア率（初回供血者集団における HCV 抗体陽性率に 70% を乗じた値を HCV キャリア率と読み替えている）は，20 歳以下ではいずれの地域も 0.1% 以下のきわめて低い値を，また肝発癌年齢と考えられる 60 歳以上の高年齢集団では，関東以西の地域でとくに高い値を示す傾向がある．年齢階級とキャリア率の関係は，地域により若干のキャリア率の高低差が認められるものの，その傾向は全国と同様であることが明らかとなっている．
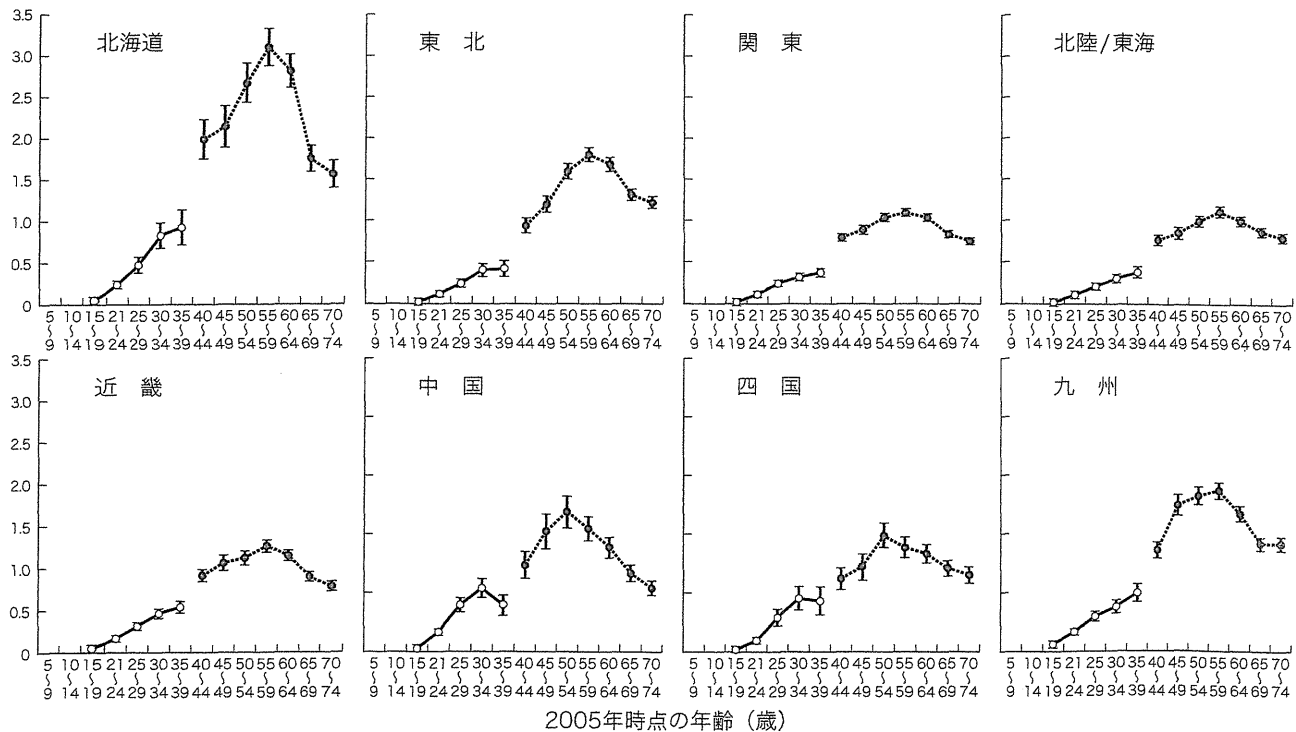
HCVキャリア率（%）

HBVキャリア率（%）

2005年時点の年齢（歳）
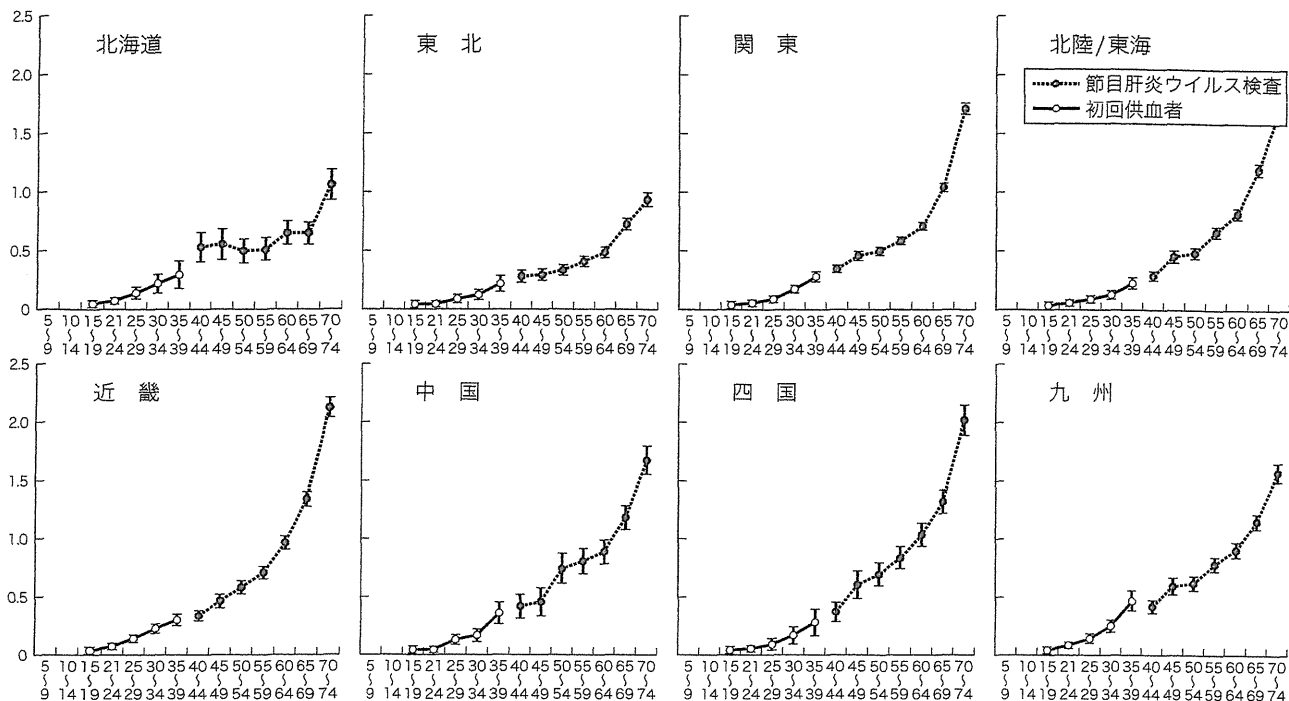
Fig. 4.　地域別年齢階級別にみた HCV・HBV キャリア率

Table 1. 肝炎ウイルスキャリア対策

a．（感染を知らないまま）潜在しているキャリア	
・肝炎ウイルス検査	検査の必要性 検査の機会の拡大（無料検査・出前検査） 対象者の拡大
b．患者としてすでに通院・入院しているキャリア	
・治療 ・治療効果などの情報提供 ・治療連携	医療費補助の運用 適切な治療への導入 専門医への受診 肝癌早期発見・治療プロトコール
c．（感染を知ったが）継続的な受診をしないままでいるキャリア	
・受診への動機づけ ・公費助成により見出されたキャリアの健康管理	現状把握と要因分析 医療機関受診率の把握 肝炎診療ネットワークへの連携
d．新規感染によるキャリア	
	感染予防対策 ワクチン キャリアの新規発生状況の把握と対策

感染を知らないまま潜在しているキャリア数の把握と肝炎ウイルスキャリア対策○

前項に示した 2 つの大規模集団を元に得た年齢階級別 HBV・HCV キャリア率と国勢調査人口を用いて，わが国におけるキャリア数の推計を行ったところ，2005 年時点では HBV キャリア推計数は，903,145 人（95% CI：83.7-97.0 万人），HCV キャリア推計数は，807,903 人（95% CI：68.0-97.4 万人）となった[4]．この推計値，HBV キャリア数約 90 万人，HCV キャリア数約 81 万人は，検査前には自身が感染を知らなかった献血集団や肝炎ウイルス検診受検者集団におけるキャリア率を元に算出された数値であることから考えると，「感染を知らないまま潜在しているキャリア」の推計数に相当する．

社会における存在状態により肝炎ウイルスキャリア（肝炎ウイルスの持続感染状態にある人）を分類すると，「a．感染を知らないまま潜在しているキャリア」，「b．患者としてすでに通院・入院しているキャリア」，「c．感染を知ったが受診しないで

いる，あるいは継続受診にいたっていないキャリア」，「d．新規感染によるキャリア」と大きく 4 分類される（Table 1）．わが国の肝炎ウイルスに持続感染しているキャリア数の全体を把握するためには，さらに「b」，「c」，「d」それぞれの数の把握（burden）が必要である．その大きさと社会における存在状態に応じて具体的なキャリア対策を講じることが効果的と考えられ，把握するための大規模調査や研究が行われているところである．

今後の肝炎ウイルスキャリア対策，ひいては肝癌対策として，「d．新規感染によるキャリア」に対しては，肝炎ウイルスの新規感染の動向調査・従来の感染防止対策を継続すること，「a．感染を知らないまま潜在しているキャリア」に対しては，肝炎ウイルス検査の必要性を周知し，家族を含んだ職域集団などの対象者の拡大を図り，対象集団ごとの検査機会の利便性を促進すること，「b．患者としてすでに通院・入院しているキャリア」に対しては，肝炎治療に適した医療へのアクセス状況，最新の抗ウイルス療法の治療効果や肝癌早期発見のための検査プロトコールなどの情報提供の現

状，医療費補助の運用と効果の把握をすること，さらに「c. 感染を知ったが受診しないままでいるキャリア」に対しては，その現状把握と要因分析を行うために，公費助成により見出されたキャリアの健康管理や医療機関受診状況の追跡調査を行うこと，が重要と考えられる[5].

おわりに○

わが国の社会生活全般における肝炎ウイルス感染の発生要因が徐々に減少し，若い世代におけるHBV キャリア率や HCV キャリア率は低い値を示すにいたっている．肝炎対策基本法(2009 年 12 月)を基盤として，すでに感染しているキャリアへの対策，具体的には，肝炎ウイルス検査の推進，肝疾患診療ネットワークの構築，新規治療法の開発などが積極的に進められている．

肝炎・肝癌対策をその病因論的また疫学的視点から捉えた場合，これまで行ってきた肝炎ウイルス感染の動向調査・感染防止対策を継続しつつ，

社会における肝炎ウイルスキャリアの存在状態別にそれぞれの課題を掲げて具体的な対策を推進することが肝癌対策にとっても重要であるといえる．肝炎対策の先進国であるわが国は，肝癌対策の新たな局面を迎えていると考えられる．

文　献○

1) 厚生労働省大臣官房統計情報部：平成 21 年人口動態統計，上巻，2009
2) 日本肝癌研究会：第 5 回～第 18 回全国原発性肝癌追跡調査報告，日本肝癌研究会事務局，1982-2009
3) 田中純子ほか：肝炎ウイルス検診受診者(2002.4-2007.3受診群)を対象とした解析．平成 19 年度厚生労働省科学研究費補助金肝炎等克服緊急対策研究事業「肝炎状況・長期予後の疫学に関する研究」報告書，p1-6, 2008
4) Tanaka J et al：Total numbers of undiagnosed carriers of hepatitis C and B viruses in Japan estimated by age- and area-specific prevalence on the national scale. Intervirology 54：185, 2011
5) 田中純子：肝炎ウイルス感染状況・長期経過と予後調査及び治療導入対策に関する研究．厚生労働省肝炎等克服緊急対策研究事業「肝炎ウイルス感染状況・長期経過と予後調査及び治療導入対策に関する研究」平成 22 年度　総括報告書，p1-27, 2011
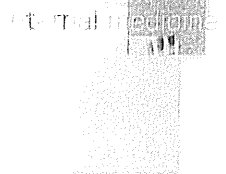
ORIGINAL ARTICLE

Residual risk of transfusion-transmitted hepatitis B virus (HBV) infection caused by blood components derived from donors with occult HBV infection in Japan

Rikizo Taira, Masahiro Satake, Shun'ya Momose, Satoru Hino, Yoshiharu Suzuki, Hiroyuki Murokawa, Shigeharu Uchida, and Kenji Tadokoro

BACKGROUND: Nucleic acid amplification testing (NAT) for hepatitis B virus (HBV) during blood screening has helped to prevent transfusion-transmitted HBV infection (TT-HBV) in Japan. Nevertheless, 4 to 13 TT-HBV infections arise annually.

STUDY DESIGN AND METHODS: The Japanese Red Cross (JRC) analyzed repository samples of donated blood for TT-HBV that was suspected through hemovigilance. Blood donations implicated in TT-HBV infections were categorized as either window period (WP) or occult HBV infection (OBI) related. In addition, we analyzed blood from 4742 donors with low antibody to hepatitis B core antigen (anti-HBc) and antibody to hepatitis B surface antigen (anti-HBs) titers using individual-donation NAT (ID-NAT) to investigate the relationship between anti-HBc titer and proportion of viremic donors.

RESULTS: Introduction of a more sensitive NAT method for screening minipools of 20 donations increased the OBI detection rate from 3.9 to 15.2 per million, while also the confirmed OBI transmission rate increased from 0.67 to 1.49 per million. By contrast the WP transmission rate decreased from 0.92 to 0.46 per million. Testing repository samples of donations missed by minipools of 20 donations NAT showed that 75 and 85% of TT-HBV that arose from WP and OBI donations, respectively, would have been interdicted by ID-NAT. The ID-NAT trial revealed that 1.94% of donations with low anti-HBc and anti-HBs titers were viremic and that anti-HBc titers and the frequency of viremia did not correlate.

CONCLUSIONS: The JRC has elected to achieve maximal safety by discarding all units with low anti-HBc and anti-HBs titers that account for 1.3% of the total donations.

The prevalence of hepatitis B virus (HBV) surface antigen (HBsAg) in Japan is slightly higher than the average for developed countries. A recent screening of blood donors, local residents, and school pupils found an estimated national prevalence of HBsAg of 0.71%.[1] However, the prevalence was higher during the 1990s, being 1.5% among first-time blood donors aged in their 40s.[2] Taking into account horizontal transmission and a birth cohort effect, a relatively large cohort with historical HBV infection might persist among older individuals in Japan.

To prevent transfusion-transmitted HBV (TT-HBV) infection, Japanese Red Cross (JRC) blood centers introduced HBsAg screening for all blood donations in 1972. In 1989, antibody to hepatitis B core antigen (anti-HBc) testing was introduced to exclude donations by people with prior HBV infection. Because total elimination of anti-HBc–reactive donations might have seriously reduced the blood supply, donations with high antibody to hepatitis B surface antigen (anti-HBs) titers and those

with low anti-HBc titers have been accepted, and only donations with low anti-HBs and high anti-HBc titers were excluded.

In addition to this serologic screening algorithm, the JRC implemented multiplex nucleic acid amplification testing (NAT) for HBV, hepatitis C virus (HCV), and human immunodeficiency virus Type 1 (HIV-1) in 1999.[3] Although NAT has greatly reinforced blood safety regarding TT-HBV infection, 4 to 13 TT-HBV infections continue to arise annually. While some occur as a result of transfusion with blood components obtained during the window period (WP), others arise due to components being derived from donors with occult HBV infection (OBI) defined as detectable HBV DNA in peripheral blood but no detectable HBsAg.[4,5] Although donations from donors with OBI have helped to maintain an adequate blood supply, such donations have also raised a concern about the risk of TT-HBV. Here, we describe the current status of TT-HBV under the NAT screening system as well as problems inherent in the current HBV screening algorithm, especially with regard to OBI-derived blood donations. We also discuss the feasibility of strategies that could increase HBV safety in countries such as Japan with a slightly elevated prevalence of HBV.

MATERIALS AND METHODS

Screening donated blood at JRC blood centers

The JRC blood centers are the only facilities authorized to handle blood collection, processing, testing, and delivery in Japan. Donated blood is screened at these centers for HBsAg, anti-HCV, anti-HIV-1 and -2, anti-human T-lymphotropic virus type 1, anti-*Treponema pallidum*, and human parvovirus B19 antigen. Whereas HBsAg-positive blood is rejected, HBsAg-negative samples are further tested for anti-HBc and anti-HBs (Table 1). Blood

---

TABLE 1. HBV screening algorithm applied at JRC blood centers*

	Anti-HBc titer	
	Low <2⁶(2⁵) or S/CO≥1.0 but <12.0	High ≥2⁶(2⁵) or S/CO ≥12.0
Anti-HBc reactive 4.9% 261,000, *49,000*		
Anti-HBs ≥ 200 IU/L	Accepted 2.04% 108,000, *20,000*	Accepted 1.38% 73,000, *14,000*
Anti-HBs < 200 IU/L	Accepted 1.31% 69,000, *13,000*	Rejected 0.19% 10,000, *2,000*

* HBsAg-negative donations are tested for both anti-HBc and anti-HBs. Dilution factors for anti-HBc titers were applied for agglutination testing. Dilution factors in parentheses were applied between 1997 and 2007. The S/CO ranges are currently used for CLEIA. Ratios (%) of donations for each category are shown (2010 data). Observed number and number per million (italics) of donations are also included.

---

with a high anti-HBs titer (≥200 IU/L) is accepted irrespective of the anti-HBc titer and that with a low anti-HBc titer is also accepted irrespective of the anti-HBs titer. Blood with high anti-HBc and low anti-HBs titers (<200 IU/L) is disqualified. All blood had been serologically tested before 2008 using the agglutination method with the initial cutoff for a high anti-HBc titer being a dilution factor of 2⁶, which was later revised to 2⁵. All agglutination tests were replaced with chemiluminescence enzyme immunoassays (CLEIAs, CL4800 testing system, Fujirebio, Tokyo, Japan) in 2008 and the threshold for anti-HBc positivity is currently a signal-to-cutoff ratio (S/CO) of 12.0. This value was validated as being essentially equivalent to an agglutination titer of 2⁵. Blood donations with elevated serum alanine aminotransferase (>60 IU/mL) are also rejected.

NAT

Samples that were qualified by the testing algorithm for anti-HBc and anti-HBs described above as well as by HBsAg testing are then screened using NAT. The JRC started NAT in 1999 using a real-time multiplex polymerase chain reaction system with a minipool format that originally comprised 500 samples (Ampli-NAT MPX system, Roche, Indianapolis, IN).[6] The pool size was decreased to 50 in 2000 and to 20 in 2004. The JRC implemented the Roche TaqScreen MPX system for NAT in 2008 with a pool size of 20, but with an approximately threefold increase in sensitivity because of the increased sample volume required for nucleic acid extraction and improvements in reagents. The screening sensitivity of HBV is 650, 260, and 76 copies/mL (50% limit of detection [LOD]; JRC data) for 50- (50p) and 20- (20p) sample pools using AmpliNAT and 20p using TaqScreen, respectively.

A trial screening using individual-donation NAT (ID-NAT) proceeded at the Tokyo Blood Center between December 2010 and May 2011 to verify the distribution of the rate of donations containing HBV DNA relative to anti-HBc titers and the residual TT-HBV risk that could arise from transfusion with blood donations that have low anti-HBc and anti-HBs titers. All available donations with both an anti-HBc titer between 1.0 and 12.0 S/CO and an anti-HBs titer of less than 200 IU/L were screened by ID-NAT using the Roche TaqScreen MPX system with a 50% LOD of 3.8 copies/mL (JRC data). The sensitivity of ID-NAT used in lookback studies (described below) was 13 copies/mL (50% LOD) using AmpliNAT until July 2008 and 3.8 copies/mL (50% LOD) using TaqScreen from August 2008.

Hemovigilance system

The JRC established a hemovigilance system in 1993 and has since collected reports on adverse effects caused by blood transfusion. Through blood screening the JRC obtains information about repeat donors who have

recently acquired infection.[7] Their previous donations are evaluated for transfusion-transmitted infection (TTI) risk by considering donation timing and performing ID-NAT on repository samples (lookback studies). If they are judged as harboring a TTI risk, the JRC notifies the relevant facilities that used the component at risk and requests that physicians investigate whether any patient who received a transfusion of the component has acquired the corresponding infection.

The JRC also obtains information about TTI in transfused patients through voluntary reports by physicians who are involved in blood transfusion at medical facilities.[7] Upon receiving such information, the JRC analyzes repository blood samples obtained from implicated donations using ID-NAT. The TTI risk of cocomponents derived from the implicated and previous donations provided by implicated donors is assessed. The JRC notifies the relevant medical facilities of the findings. Implicated blood components are interdicted if they have not yet been used for transfusion.

The JRC headquarters and central laboratory determine the causal relationship between the implicated donation and posttransfusion infection considering patient clinical course, results of virologic analysis including ID-NAT and sequence analysis, serologic viral markers, and donation timing. Even if all repository samples implicated for TTI are verified as being ID-NAT-negative, implicated donors are followed up for repeat donation thereafter for sero- or NAT conversion, because the possibility that the index donation was provided during the ID-NAT WP persists. All processes for lookback studies are defined in national guidelines[8] that describe in detail the test items and timing of testing for donated blood and at-risk patients in addition to the roles of the relevant physicians, blood centers, and blood authority.

Sequence analysis

The HBV genome sequence identity is assessed between implicated repository blood samples and patient samples by sequencing 1550 bp of the alpha region within the HBV pre-S and S regions using a genetic analyzer (ABI 3130XL, Life Technologies Japan, Tokyo, Japan). When the viral load is too low to sequence, viral nucleic acid is further extracted from larger plasma volumes if the accompanying plasma bag is available. When findings are ambiguous, HBV obtained from donor or patient samples is cloned, amplified, and sequenced.

Estimation of current risk of TT-HBV

Although universal pre- and posttransfusion testing of patient samples for TTI has been recommended, the likelihood that all transfused patients undergo this evaluation is low. Moreover, the JRC hemovigilance system described

above is voluntary. Therefore, TTI might be underreported to JRC blood centers. The exact amount of TT-HBV infections that could occur under the current screening system must be defined to assess novel TT-HBV–mitigating strategies. This study therefore reevaluated the current risk of TT-HBV infection based on data obtained under current system.

The projected number of ID-NAT–positive donations derived from OBI donors was calculated using the ID-NAT positivity rate obtained in the ID-NAT trial screening described above and the number of donations with low anti-HBc and anti-HBs titers. The additional WP yield in donations determined by ID-NAT was calculated based on rates of detection of recently infected donors.[9,10] Assuming that the frequency of donation is constant at any time during the presymptomatic phase of acute infection, the yields by tests for an infection marker are in direct proportion to the length of time during which each test gives a yield. The potential ID-NAT yield (screening NAT negative) was calculated herein by multiplying the screening NAT yield by the ratio of the interval between ID-NAT detection and 20p-NAT detection (11.2 days) to that between 20p-NAT detection and HBsAg detection (9.7 days). The interval covered by each NAT strategy (11.2 and 9.7 days) was calculated using the value for the detection limit of each test (3.8, 76, and 1000 copies/mL for ID-NAT, 20p-NAT, and CLEIA detection, respectively) and the doubling time of HBV in human peripheral blood (2.6 days).[9,11] The number of donations that could appear in the ID-NAT–negative WP was similarly calculated separately for each component type taking into account both the interval between 1 copy/bag and ID-NAT detection deduced from the mean plasma volume of each component type and the number of each component issued to hospitals.

We estimated the number of TT-HBV infections with reference to our previous systematic lookback study.[7] The infectivity of ID-NAT–positive and screening NAT-negative components was calculated in that study as being 3% (95% confidence interval [CI], 0%-17.2%, n = 33) and 50% (95% CI, 28.2%-71.8%, n = 22) for OBI- and WP-derived components, respectively. The incidence rate for TT-HBV infections was thus obtained by multiplying the number of estimated at-risk donations deduced using the above method by the infection rates (0.03 or 0.5).

Statistical analysis

Data were statistically analyzed using computer software (SSRI for Windows, Excel Statistics Version 8, Social Survey Research Information Co. Ltd, Tokyo, Japan). Significance was determined using the chi-square test except for associations between total viral load in the components and alanine aminotransferase (ALT) levels in patients that were evaluated using the Mann-Whitney U test.

RESULTS

Reports of possible TT-HBV infections

The JRC blood centers received 789 reports of possible TT-HBV infections between 2001 and 2010 (Fig. 1). The number of such reports obviously increased in 2004 and 2005 because a nationwide systematic retrospective study started in 2004 that also identified patients with TT-HBV infection that would have previously been unrecognized. Causality was investigated in all but two of these possible TT-HBV infections. The possibility of TT-HBV infection was precluded in 97 (12.3%) of the 789 reported patients without testing repository samples based on evaluation of the patient's clinical course and the transfusion setting for each. For all of the remaining patients, repository samples were tested serologically and by ID-NAT to detect the HBV genome. Of the 789 initial reports, 98 (12.4%) were

Fig. 1. Annual number of potential TT-HBV infections. (☐) Patients in which possibility of TT-HBV was excluded (n = 97, 12.3%) without testing repository samples. (■, ☐) Patients in whom HBV DNA was identified or not, respectively, in the repository samples corresponding to a donation from the donor of the implicated blood components. Four patients are not included as HBV DNA sequence identity was not established.

determined to be TT-HBV infections after the introduction of 50p-NAT. The HBV sequence identity was established between donor and recipient in 88 of these cases, and TT-HBV was determined considering other HBV markers and the clinical setting in the remaining 10. An HBV genome was not detected in repository samples for 587 (74.4%) potential TT-HBV infections. Although HBV was detected in four repository samples, HBV sequence identity was not confirmed between donors and recipients. Forty-two (43%) of the established TT-HBV infections were discovered through lookback studies that were started based on risk information provided by JRC blood centers. The remaining 56 (57%) were initially recognized by physicians at medical facilities. The number of established TT-HBV infections ranged from 4 to 13 per year between 2006 and 2010.
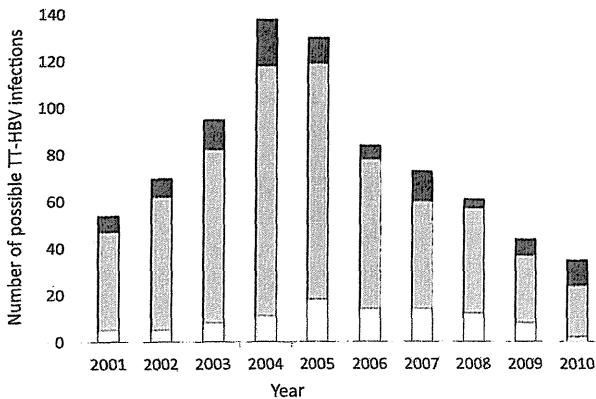
Infection status of donors implicated in TT-HBV infection

The sensitivity of NAT screening improved through the three phases described above (50p-AmpliNAT, 20p-AmpliNAT, and 20p-TaqScreen). With the increased sensitivity of 20p-TaqScreen, the NAT yield of OBI donations increased from 3.9/million to 15.2/million, whereas the yield of WP donation decreased from 13.2/million to 5.7/million (Table 2). This was caused by the simultaneous introduction of CLEIA in 2008 for serologic screening including HBsAg detection, which effectively shortened the period that could be covered by 20p-NAT.

The established TT-HBV infections that occurred during each period were categorized based on the presence or absence of the HBV genome in the implicated component (that is, ID-NAT positive or negative) and the infection status of the donation (WP related [anti-HBc nonreactive] or OBI related [anti-HBc reactive]). Table 2 also shows the numbers of established TT-HBV infections associated with each group during each period. Figure 2 shows the incidence (per million donations) of estab-

TABLE 2. NAT yield and number of TT-HBV infections relative to three phases of screening NAT*			
Screening system	50p-AmpliNAT	20p-AmpliNAT	20p-TaqScreen
Duration of screening period	Feb. 2000–Jul. 2004 (4.5 year)	Aug. 2004–Jul. 2008 (4.0 year)	Aug. 2008–Mar. 2010 (1.67 year)
Sensitivity of screening NAT (copies/mL)†	650	260	76
Sensitivity of ID-NAT used for lookback study (copies/mL)†	13	13	3.8
Number of donations tested	24,702,784	19,513,054	8,746,037
Confirmed WP donations (/million)	473 (19.1)	258 (13.2)	50 (5.7)
Confirmed OBI donations (/million)		76 (3.9)	133 (15.2)
Number of donations causing established HBV transmission			
ID-NAT–negative WP	5	6	1
ID-NAT–positive WP	28	12	3
ID-NAT–negative OBI	4 (1)‡	1 (0)‡	2 (1)‡
ID-NAT–positive OBI	13 (1)‡	12 (1)‡	11 (5)‡

* Yields by ID-NAT trial conducted from December 2010 are not included in the table.
† 50% LOD.
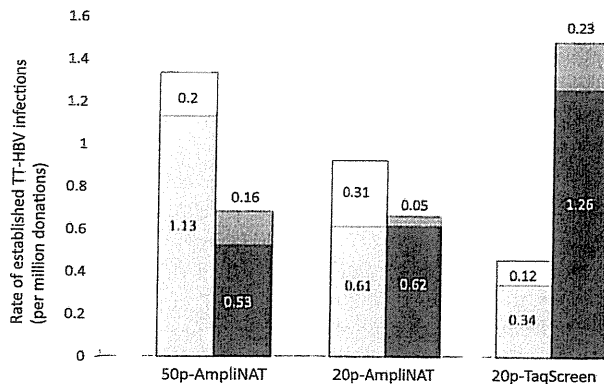‡ Numbers of donations with anti-HBs of greater than 10 mIU/mL.

Fig. 2. Number of established TT-HBV infections grouped according to pool-based NAT screening systems. See Table 2 for intervals when indicated NAT systems were applied, sensitivities of NAT systems used, and actual yields for each category at each interval. (□, ▣) Infections caused by transfusion with ID-NAT–negative and -positive WP-derived components, respectively. (▨, ■) Infections caused by transfusion with ID-NAT–negative and -positive OBI-derived components, respectively.

lished TT-HBV infections relative to the three periods. The rate of infections caused by transfusion with a WP-derived component notably decreased with increasing NAT sensitivity, but that caused by transfusion with OBI-derived components rather increased despite the increased NAT sensitivity. Current NAT screening protocols indicated that TT-HBV infections occur more frequently due to transfusion with OBI- than with WP-related components (1.49/million vs. 0.46/million donations; Fig. 2). Nine TT-HBV infections occurred as a result of transfusion with blood components containing more than 10 mIU/mL anti-HBs during the past decade (Table 2). Two of them were caused by donations with negative ID-NAT.

The number of TT-HBV infections caused by transfusion with ID-NAT–negative components accounts for 15% (2/13) and 25% (1/4) of OBI- and WP-related TT-HBV infections, respectively, according to the current NAT system (Table 2). These infections involving ID-NAT–negative donations were determined as TTI by analyzing repository blood samples obtained before the index donation and/or by following up with the implicated donors after the index donation. Details of the clinical course of a typical TT-HBV infection caused by ID-NAT–negative OBI-related blood components are shown in Tables 3 and 4.

Impact of blood product on transmission rate

Table 5 shows the numbers of implicated donations by either ID-NAT negative or positive for groups categorized by the type of component and WP/OBI status. During the past decade, ID-NAT–positive donations have caused 79 TT-HBV infections. Transfusion with red blood cells (RBC),

fresh-frozen plasma (FFP), and platelet concentrate (PC) was associated with infections in 42, 22, and 15 of them, respectively. Of 19 TT-HBV infections associated with ID-NAT–negative donations, 2, 4, and 13 were caused by transfusion with RBCs, FFP, and PC, respectively. Transfusion with blood components containing a larger plasma volume (FFP and PC, but not RBCs) caused more frequent TT-HBV infections among patients who received ID-NAT–negative donations (17/19, 89%) than among those who received ID-NAT–positive donations (37/79, 47%; $p < 0.01$), which could be a reflection of the plasma volume effect on infectivity.

Table 5 also shows that if ID-NAT had been implemented during the screening, 81% of established TT-HBV infections would have been avoided. The introduction of ID-NAT would have been the most (95%) and least (54%) effective for preventing TTI caused by RBC- and PC-related transfusions, respectively. Under the current 20p-TaqScreen system, 75 and 85% of TT-HBV infections arising from WP and OBI donations, respectively, are ID-NAT positive and will be interdicted by ID-NAT. In particular, the effect of ID-NAT will be 100% for OBI-related infections caused by RBC transfusion.

Outcomes of patients with TT-HBV infection

ALT levels during TT-HBV infection were determined in 68 transfusion recipients who developed TT-HBV infection. Table 6 shows the maximal ALT values relative to WP or OBI donations and ID-NAT–positive or -negative donations. Almost half (47%, 32/68) of the patients had maximal ALT values of more than 1000 IU/L. The proportion of patients with maximal ALT of more than 1000 IU/L was greater in OBI-related (61%, 19/31) than in WP-related (35%, 13/37; $p < 0.05$) infections. Total viral load in the implicated components did not significantly differ between patients with ALT values above and below 1000, which was true for both WP- and OBI-related infections. Although barely insignificant, total infused viral load tended to be lower in OBI- than in WP-related patients among groups with maximal ALT values of more than 1000.

Three patients with TT-HBV infection died of fulminant hepatitis after the introduction of NAT. One was caused by transfusion with PC derived from an ID-NAT–negative, WP-related donation (Genotype A with wild type precore region). The other two developed hepatitis after transfusion with RBCs derived from ID-NAT–positive, OBI-related donations (Genotype B with a G1898A precore mutation and Genotype C with a G1896A precore mutation).

ID-NAT screening trial in donations with low anti-HBc and anti-HBs titers

During a 6-month ID-NAT trial, 4742 (0.74%) of 640,628 blood donations at the Tokyo Blood Center with low anti-

HBc and anti-HBs titers were analyzed by ID-NAT. The number of donations analyzed by ID-NAT decreased as the anti-HBc titer increased (Fig. 3). HBV DNA was detected in 92 (1.94%) of the 4742 donations. Figure 4 shows the frequency of ID-NAT–positive donations relative to the anti-HBc titer. The frequency of ID-NAT positivity for HBV did not correlate with the anti-HBc titer and did not tend to increase with an increasing anti-HBc titer. The proportions of anti-HBs–positive (>10 mIU/mL) donations among those that were ID-NAT positive and

negative were 77 and 75%, respectively, and did not significantly differ. The proportion of anti-HBs–positive donations increased with increasing anti-HBc S/CO values among ID-NAT–negative donations (67.5, 82.0, and 87.5% for anti-HBc S/CO 1.0-3.9, 4.0-7.9, and 8.0-11.9, respectively; p < 0.01 between any two groups). The frequency of ID-NAT positivity between males (1.8%) and females (2.4%) did not significantly differ. Eighty-three (90.2%) of the 92 ID-NAT–positive donors were at least 50 years of age. Fifteen had a viral load of less than 100 copies/mL, whereas quantitative NAT could not detect HBV DNA loads in samples from the remaining 77. The distribution of HBV genotypes among the ID-NAT–positive donations did not differ from that among the general Japanese population: Genotypes A, B, C, and D, n = 1, 24, 45, and 1, respectively (21 were undetermined).

Estimation of current TT-HBV risk in Japan

From the frequency of ID-NAT–positive (1.94%) donations among those with low anti-HBc and anti-HBs titers (69,000/year or 13,000/million; see Table 1 and below), we calculated that 1339/year or 252/million donations should be ID-NAT positive among screening NAT-negative donations with low anti-HBc and anti-HBs titers. Using an infectivity rate of 3%[7] among components derived from OBI donations that were screening NAT negative and ID-NAT positive, we calculated that 40/year or 7.6/million OBI-related TT-HBV infections should arise. If TT-HBV infections related to OBI-derived ID-NAT-negative donations are taken into account, then the total number of TT-HBV infections should be 47/year or 8.9/million. This estimate was based on the observation that TT-HBV infection caused by ID-NAT–negative components during the

TABLE 3. Representative TT-HBV infection caused by OBI-derived, ID-NAT–negative blood component: clinical course of a patient who received an implicated blood component.

Date	Clinical events and test results
Nov. 10, 2008	Surgery to treat head injury*
	HBsAg negative, anti-HBc negative, HBV DNA negative, preoperatively
	Transfused until Nov. 20 with 21 RBC units, 5 PCs, and 11 FFP† including one derived from the donation of Mar. 27, 2008, shown in Table 4
Mar. 05, 2009	AST 15, ALT 32
Mar. 25, 2009	AST 517, ALT 1273
Mar. 30, 2009	AST 1312, ALT 3110, HBsAg positive, IgM-anti-HBc positive
	Reported to JRC blood center
Mar. 31, 2009	AST 695, ALT 2396
Apr. 01, 2009	HBsAg negative, anti-HBs positive, HBV DNA positive

* Recipient was a teenage boy who was injured in a traffic accident.
† HBV DNA was not detected based on ID-NAT for the repository samples from these 37 blood components transfused. These results were obtained in the first lookback study performed in April 2009.

TABLE 4. Representative TT-HBV infection caused by OBI-derived, ID-NAT–negative blood component: HBV marker profile of blood donor responsible for the outcome shown in Table 3

Date of donation	Date of testing	Test results
Oct. 17, 2007*	Oct. 17, 2007 (screening)	Pool NAT negative, anti-HBc 2⁴ (negative), anti-HBs negative
	Feb. 24, 2010 (repository sample tested in second lookback study)	ID-NAT negative
Mar. 27, 2008† (index donation)	Mar. 27, 2008 (screening)	Pool NAT negative, anti-HBc 2⁴ (negative), anti-HBs negative
	Apr. 7, 2009 (repository sample tested in first lookback study)	ID-NAT negative (negative result reported to corresponding facility)
Feb. 05, 2010‡	Feb. 05, 2010 (screening)	Pool NAT negative, anti-HBc 15.4 S/CO§ (positive), anti-HBs negative
	Feb. 10, 2010 (donated blood sample tested in second lookback study)	ID-NAT positive (high probability of TT-HBV infection in Patient A reported to corresponding facility)

* RBCs derived from this donation were transfused to an HBsAg-negative patient. Patient continued to be HBsAg-negative until May 2008 when he died.
† FFP derived from this donation was transfused to patient shown in Table 3. Cocomponent (RBCs) processed from this donation was transfused to a patient who died of the primary disease soon after transfusion. Whether TT-HBV occurred remains unknown.
‡ This donation was rejected due to anti-HBc seroconversion and a second lookback study was conducted on the donation of October 17, 2007.
§ Because of very low HBV load in donated blood sample of February 5, 2010, HBV sequence was assessed in donor blood only at 193 bp (Nucleotides 475-667) of S region. HBV sequence in that region was identical except for nt. 654 between the blood samples from donor and patient on April 01, 2009.

TABLE 5. Blood components implicated in established TT-HBV infection*

| | ID-NAT+/ID-NAT− | | | | | |
| | WP transmissions established | | | OBI transmissions established | | |
Screening period	RBCs	FFP	PC	RBCs	FFP	PC
50p-AmpliNAT	15/0	6/1	7/4	5/0	5/1	3/3
20p-AmpliNAT	8/2	0	4/4	7/0	5/1	0
20p-TaqScreen	2/0	0	1/1	5/0	6/1	0/1
Total	25/2	6/1	12/9	17/0	16/3	3/4

| | WP plus OBI | | | | All components | | |
	RBCs	FFP	PC	FFP + PC	WP	OBI	Total
50p-AmpliNAT	20/0	11/2	10/7		28/5	13/4	41/9
20p-AmpliNAT	15/2	5/1	4/4		12/6	12/1	24/7
20p-TaqScreen	7/0	6/1	1/2		3/1	11/2	14/3
	100%	86%	33%		75%	85%	82%
Total	42/2	22/4	15/13	37/17	43/12	36/7	79/19
	95%	85%	54%		78%	84%	81%

* Ratios (%) in the two bottom rows represent rates of ID-NAT–positive events or effectiveness of ID-NAT implementation.

TABLE 6. Maximal values for ALT in patients with TT-HBV infection and total viral load contained in implicated components

| | ALT | |
	<1000	>1000
WP* (n‡)	24 (7)§	13
OBI† (n‡)	12 (2)§	19
Total viral load (copies/bag)‖		
WP		
n‡	21	10
Min	40	100
Max	260,000	560,000
Median	1,400	9,100
Mean	20,460	74,790
OBI		
n‡	8	16
Min	60	40
Max	6,240	19,200
Median	630	1,470
Mean	1,440	3,750
ID-NAT status		
Positive‡	30	28
Negative‡	6	4
Component types		
RBCs‡	19	16
FFP‡	7	13
PC‡	10	3

* Patients transfused with WP-related components include 11, 8, and 18 patients with malignant hematologic disorder, solid tumor, and others, respectively.
† Patients transfused with OBI-related components include 7, 11, and 13 patients with malignant hematologic disorder, solid tumor, and others, respectively.
‡ Numbers of patients.
§ Numbers in parentheses, patients with maximal ALT values of less than 100 IU/L.
‖ Total viral load was calculated using viral concentrations in implicated donations and average plasma volume of each component type. When viral load was less than 100 copies/mL, total viral load in the component was calculated assuming that viral concentration is logarithmically distributed between 1 and 100 copies/mL.
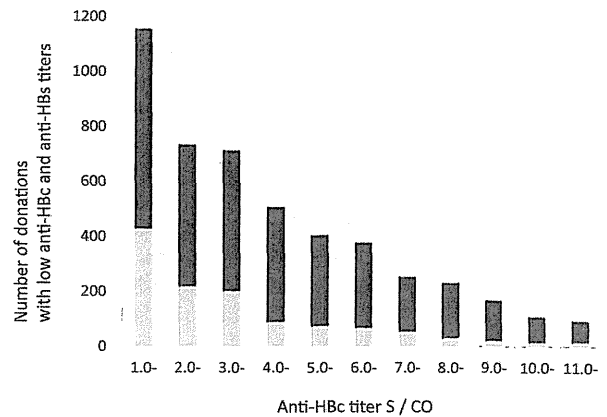

Fig. 3. Number of donations screened by ID-NAT trial categorized by anti-HBc titer. All donations tested had low anti-HBc (S/CO 1.0-11.9) and anti-HBs (<200 IU/L) titers and were qualified serologically based on algorithm applied at JRC blood centers. Donations verified to be ID-NAT-positive were disqualified. (■, ▢) donations with anti-HBs titers of more than and not more than 10 mIU/mL, respectively.

20p-TaqScreen period accounted for 15% (2/13) of all OBI-related infections (Table 2).

We estimated how many more WP-related TT-HBV infections would be prevented by introducing ID-NAT. The current screening NAT yield (30 donations/year or 5.7/million, Table 2) was multiplied by the ratio of the interval between ID-NAT and 20p NAT detection (11.2 days) to that between 20p NAT and HBsAg detection (9.7 days). We then deduced that 34.6/years or 6.6/million more viremic donations would be captured by ID-NAT. The number of ID-NAT–negative WP donations was calculated separately for each component type. Based on the plasma volume of each component (20, 200, 240, 450, and 120 mL for RBCs, PC, FFP-3, FFP-5, and FFP1.5,
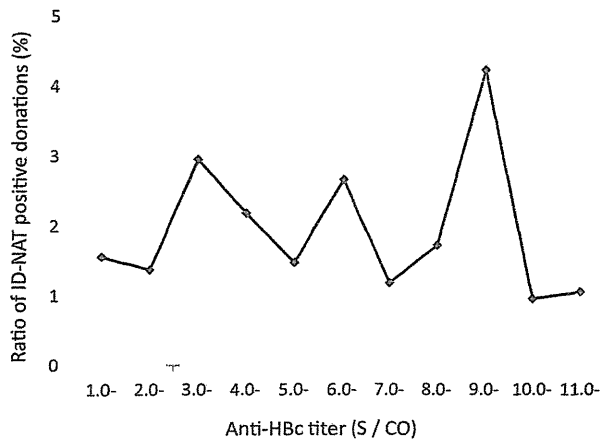
Fig. 4. Ratios (%) of ID-NAT–positive donations with low anti-HBc and anti-HBs titers relative to anti-HBc titer.

respectively), the deduced intervals between 1 copy/bag and ID-NAT detection were 16.3, 24.9, 25.5, 28, and 23 days, respectively. The ratio of the number of those components issued to hospitals is 6.3:2.2:2.1:0.7:0.1. The incidence of ID-NAT–negative WP donations calculated from these data was 59.5/year or 11.4/million. Adding ID-NAT–positive WP donations (34.6/year or 6.6/million), current risk related to WP donations amounts to 94.1/year or 18.0/million. The effect of ID-NAT on the reduction of all WP donation would be 37% (6.6/18.0). If the infectious risk (50%) of ID-NAT–positive, screening NAT-negative WP-related components is also applied to ID-NAT–negative WP-related components, the total number of WP-related TT-HBV infections would be 47.1/year or 9.0/million. Together, these estimates for WP- and OBI-related TT-HBV infections indicate that 94.1/year or 17.9/million TT-HBV infections are likely to occur in Japan.

DISCUSSION

Infection with HBV results in a wide spectrum of clinical manifestations ranging from asymptomatic liver dysfunction with only slightly elevated transaminase levels or acute self-limiting hepatitis to chronic hepatitis that in some patients progresses to cirrhosis, liver failure, or hepatic cell carcinoma. In rare cases, HBV infection can cause fulminant hepatitis that is associated with high mortality. Fulminant hepatitis in Japan is frequently associated with primary infection by HBV carrying precore or core-promoter mutations.[12,13] These HBV mutants are frequently found among chronic HBV carriers[14,15] who typically have an anti-HBc–positive serostatus. To prevent fulminant hepatitis arising as a result of blood transfusion,[16] the JRC incorporated anti-HBc testing into blood screening in 1989.

The agglutination method had been used for all serologic testing at JRC blood centers before 2008. Although this method was somewhat insensitive to HBsAg, it could semiquantify anti-HBc. Thus, the cutoff point for the anti-HBc titer had been set at 2^6, and donations with an anti-HBc titer of at least 2^6 and an anti-HBs titer of less than 200 mIU/mL were disqualified.[17] Although this anti-HBc testing had essentially prevented transfusion-transmitted fulminant hepatitis since 1989,[18] reports of fulminant or acute severe hepatitis continued for an additional 7 years. These conditions were attributed to transfusion with components with a 2^5 anti-HBc titer.[19] Consequently, the JRC lowered the anti-HBc cutoff from 2^6 to 2^5 in 1997. The agglutination method for serologic screening was replaced in 2008 with CLEIAs, which can also semiquantify anti-HBc. The policy described above is maintained in the algorithm for HBV screening with CLEIA; the range defined as a low anti-HBc titer includes S/CO values between 1.0 and 11.9, and donations with anti-HBc S/CO values within this range are currently accepted.

The highly sophisticated strategy of multiplex NAT was designed to decrease the incidence of TTI. Implementing HBV NAT into blood screening was important in Japan mainly because of the unsatisfactory sensitivity of the standard agglutination method to HBsAg. The JRC implemented multiplex NAT targeting HBV DNA, HIV RNA, and HCV RNA during 1999[6] and improved the sensitivity of the test at three points. The 98 infections described herein had been confirmed as TT-HBV since the introduction of 50p-AmpliNAT in 2000. The HBV genome was not detected in donor repository samples of 587 (74.4%) suspected TT-HBV infections. The JRC has informed the appropriate physicians of the ID-NAT results of viral detection that imply a low or high probability of TT-HBV infection.

In parallel with the increase in the screening NAT sensitivity, the incidence of WP-related TT-HBV infection has decreased as predicted, whereas that of OBI-related TT-HBV infection has not decreased (Fig. 2). To explain the increasing number of OBI-related TT-HBV infections, the increase in the sensitivity of NAT used in JRC laboratories for retrospective studies might have helped to identify TT-HBV infections, thus sustaining the number of OBI-related TT-HBV infections despite improvements in screening NAT sensitivity.[4]

This consideration could encourage the speculation that most of the 587 infection reports that had been excluded from established TTI (Fig. 1) based on negative results from repository samples might have been confirmed as TT-HBV had more sensitive NAT and a larger sample volume been analyzed. With regard to this notion, the outcomes of recent hemovigilance for TT-HBV are described below. During the 20p-Taqscreen period, the JRC received 61 clinical reports of possible HBV-TTI. Seventeen were determined as TTI, among which, three

repository samples were ID-NAT negative. Historical HBV infection was confirmed in 10 patients by retesting pre-transfusion samples. Results from HBV tests of posttransfusion samples from five patients were false positive. The possibility of TT-HBV was ruled out in two patients related to ID-NAT–negative donations because repeated blood donations from two of two and three of three implicated donors were not sero- or NAT-converted. The remaining 27 patients related to ID-NAT–negative donations are inconclusive for TTI as follow-up studies have not yet been completed. Some of the 587 reported infections had been confirmed to be associated with passive anti-HBc transfer from infused components. Thus, it is unlikely that a considerable proportion of the infections excluded from the TTI category were real TT-HBV infections.

Among 19 patients with TT-HBV infections associated with ID-NAT–negative donations, 17 (89%) of them were caused by transfusion with FFP or PC that contained a larger plasma volume (120 to 450 mL) than RBCs (20 mL). In contrast, 37 (47%) were caused by FFP or PC among 79 infections associated with ID-NAT–positive donations (Table 5). This finding suggests that because HBV infectivity is extremely high, the relationship between infectivity and plasma volumes contaminated with HBV could only be established in the era of ID-NAT screening when the viral load in the donation is low enough to escape ID-NAT screening. This might explain why we could not previously establish such a relationship using viral loads around the sensitivity of the pool-based NAT system or serology.[7] If ID-NAT is introduced as routine screening, it will prevent 75 and 85% of WP- and OBI-related infections. In particular, all RBC-related TT-HBV could be prevented because of the small plasma volume involved. The finding also suggests that novel viral reduction technologies[20,21] could be an attractive strategy to decrease the incidence of TT-HBV because these technologies are presently more applicable to FFP or PC than to RBC.

The maximal ALT levels of patients with TT-HBV infection showed that transfusion with components harboring an extremely low HBV load that escaped NAT screening is not necessarily associated with mild clinical illness. This seems particularly true for OBI-related infection (Table 6). The frequency with which transfusion causes severe hepatitis (i.e., ALT > 1000) is significantly higher for OBI- than for WP-derived components. Moreover, OBI-derived components tend to cause severe hepatitis despite lower total viral loads compared with those in the WP-derived components. These findings should be further substantiated by analyzing samples from patients that are regularly obtained after transfusion because most of the maximal ALT values described in this article were found after occasional sampling.

Three patients died of TT-HBV fulminant hepatitis caused by transfusion with blood that had escaped NAT screening. Two of them were notably caused by transfusion of OBI-derived RBCs, and the other was caused by an ID-NAT–negative WP donation. Although a larger plasma volume might generally be required to establish TT-HBV infection under the NAT screening system, plasma volume or the total infused viral load might not be determining factors in fulminant hepatitis. Although viral genome mutations such as those in precore or core-promoter regions are frequently associated with the development of fulminant hepatitis in Japan,[12,13] other crucial factors have not clearly been demonstrated despite considerable investigation.

The JRC accepted 5.3 million donations in 2010, of which 4.9% (261,000) was anti-HBc reactive (Table 1), 0.19% (10,000) was rejected because of high anti-HBc and low anti-HBs titers. Another 3.4% (182,000) was accepted because of high anti-HBs titers ($\geq$200 IU/L). The notion that blood components with an anti-HBs titer of more than 100 IU/L are not infectious is generally accepted.[22-25] The relationship between anti-HBs titer and TT-HBV infection will be discussed elsewhere (manuscript in preparation). Importantly, 1.3% of donations (69,000) with low anti-HBc and anti-HBs titers were accepted, and this category included all donations to which OBI-related TT-HBV infections were attributed. Our ID-NAT trial verified that 1.94% of the donations with low anti-HBc and anti-HBs titers were HBV DNA positive.[26] Accordingly, an estimated 1339/year or 252/million viremic OBI donors and 47/year or 8.9/million TT-HBV infections caused by OBI-derived components would be missed by the current screening algorithm. When estimates for WP-related TT-HBV infections are included, the calculated number of TT-HBV infections was 94.1/year or 17.9/million. Whole blood withdrawn from donors in Japan is split into RBCs and FFP, and the total number of components processed averages 23% more than the number of donations. However, because of outdating and rejection by testing or processing problems, the number of components finally issued by JRC becomes almost the same as the number of donations. Therefore, the calculated number of TT-HBV infections was not significantly influenced by the issue of splitting.

The considerable discrepancy between the estimated and established TT-HBV incidence per million (8.9 to 1.49 and 9.0 to 0.46 for OBI-related and WP-related infections, respectively) might be due to the following factors. A clinical manifestation of HBV infection is often unclear in patients transfused with blood components harboring a low viral load and low proliferative ability. Physicians might thus be likely to overlook infection under such circumstances. Medical practitioners are not compliant with national guidelines for lookback investigations. Indeed, only 30% to 40% of transfused patients were reportedly traced for TTI even after the guidelines were established.[27] A considerable proportion of patients who receive blood

transfusions die before TTI evaluation.[28] In fact, when we inquired about the outcomes of transfusions with components containing verified HBV at medical facilities, 99 (42%) of 238 patients who had been transfused with such components had already died (JRC data from 2009 to 2010). The transmissibility of ID-NAT–positive donations might require reevaluation because of the low numbers of patients analyzed in the previous study[7] (30 and 22 for OBI- and WP-related cases, respectively). The fact that a large proportion of elderly patients are immune to HBV due to prior infection might also contribute to the low figure for established TT-HBV and, finally, anti-HBs in cotransfused components neutralizes HBV. Classified WP donation that is anti-HBs positive and could be attributed to possible vaccine breakthrough infection or anti-HBc–negative chronic OBI could also be a factor influencing infectivity. However, we have not encountered any implicated WP donations with anti-HBs among established TT-HBV infections.

Because of the high probability of a residual risk of TT-HBV, novel strategies that reinforce the safety of blood components but do not damage the blood supply should be implemented. Transfusion with ID-NAT–negative infectious components currently cause 15 and 25% of OBI- and WP-related TT-HBV infections, respectively (Table 2), and screening with ID-NAT would interdict 85 and 75% of these infections, respectively (Table 5). With respect to this, the ID-NAT screening of only donations with low anti-HBc and anti-HBs titers that are currently qualified has been suggested.[29] However, screening with ID-NAT might not be as effective as expected. For example, the variability in viral load in individuals with OBI might allow persistent OBI-related TT-HBV infection; some individuals might have an intermittently elevated viral load.[30-33] Such donations could be identified as HBV positive only when the viral load exceeds the detection threshold of ID-NAT screening. Alternatively, the detection of intermittent viremia might reflect the stochastic phenomenon inherent in NAT technology, particularly at very low viral concentrations. Moreover, one report describes a donor in whom viral load increased in blood samples over a period of several years.[34] Nine among 48 blood donations from this donor were ID-NAT positive, and two of four ID-NAT–positive and three ID-NAT–negative blood transfusions had caused TT-HBV infections. The diverse fluctuation of viremia described above has supposedly hindered the efficient detection of viremic donations by pool-based NAT screening,[35] which is predictable even in the event of ID-NAT screening. Table 5 shows that ID-NAT is not sensitive enough in 16% of established OBI-related transmission events although most of those events are caused by FFP or PC transfusions and ID-NAT screened RBC transfusions are relatively safe. Moreover, although viremia is considered undetectable in most individuals with OBI, this assumption might be dependent on the sensitivity of the NAT used; a considerable number of donations might have viremia with a viral load below the ID-NAT detection limit.

Another strategy that might increase the safety of OBI-derived donations could be to accept only those OBI-derived donations with a profile that is safer than the current standards, if such a profile can be found and systematically applied. We initially expected to find that OBI donations with a very low anti-HBc titer would be safer based on ID-NAT. However, the finding from the ID-NAT trial was that the frequency of viremia does not correlate with anti-HBc titers in the range of S/CO 1.0 to 11.9. Therefore, we concluded that the risk of TT-HBV infection will not be mitigated by implementing a strategy that qualifies only donations with very low anti-HBc titers such as S/CO between 1.0 and 3.0.

We speculated during 2003 that more than 4% of donations would be disqualified if the anti-HBc cutoff were set at 2^1, that is, if all donations with low anti-HBc and anti-HBs titers are rejected. We thought that the loss of so many donations would cause catastrophic damage to the blood inventory and thus that cutoff was not implemented. However, based on current data, the number of donations received in 2010 with low anti-HBc and anti-HBs titers was 69,000, which accounts for 1.31% of all donations in Japan. Given this ratio, we consider that to eliminate all donations with low anti-HBc and anti-HBs titers is feasible. We verified that severe hepatitis is caused more often by OBI- than WP-derived blood. The fact that two patients died of fulminant hepatitis related to OBI-related donations is also serious. Rejecting this category of donations would eliminate nearly all those harboring a risk of OBI-related infection.[26] However, a slight, but distinct risk of TT-HBV infection might persist because a small fraction of OBI donors have an anti-HBc titer of less than 1.0 S/CO, and these donors as well as NAT WP donors present a TT-HBV risk.[36] A committee of the Ministry of Health, Labour and Welfare of the Japanese government has just discussed and authorized the implementation of a new policy in which all donations with low anti-HBc and anti-HBs titers would be rejected.

In conclusion, ID-NAT screening of donations with low anti-HBc and anti-HBs titers revealed that nearly 2% of these donations were associated with low-level viremia and that viremia was identified over the entire range of anti-HBc titers. Importantly, anti-HBc titer did not correlate with the frequency of viremia. The elimination of all donations with low anti-HBc and anti-HBs titers would be important to any strategy aimed at preventing OBI-related TT-HBV infections in countries such as Japan that have a slightly elevated HBV prevalence in blood donations. If this strategy is implemented, the only acceptable donors with OBI in Japan will be those with high anti-HBs titers (≥ 200 IU/L).

REFERENCES

1. Tanaka J, Koyama T, Mizui M, Uchida S, Katayama K, Matsuo J, Akita T, Nakashima A, Miyakawa Y, Yoshizawa H. Total numbers of undiagnosed carriers of hepatitis C and B viruses in Japan estimated by age- and area-specific prevalence on the national scale. Intervirology 2011;54:185-95.

2. Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, Suzuki K, Miyakawa Y, Yoshizawa H. Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995-2000. Intervirology 2004;47:32-40.

3. Ohnuma H, Tanaka T, Yoshikawa A, Murokawa H, Minegishi K, Yamanaka R, Iizuka HY, Miyamoto M, Satoh S, Nakahira S, Tomono T, Murozuka T, Takeda Y, Doi Y, Mine H, Yokoyama S, Hirose T, Nishioka K; Japanese Red Cross NAT Screening Research Group. The first large-scale nucleic acid amplification testing (NAT) of donated blood using multiplex reagent for simultaneous detection of HBV, HCV, and HIV-1 and significance of NAT for HBV. Microbiol Immunol 2001;45:667-72.

4. Allain JP. Occult hepatitis B virus infection: implications in transfusion. Vox Sang 2004;86:83-91.

5. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, Craxì A, Donato F, Ferrari C, Gaeta GB, Gerlich WH, Levrero M, Locarnini S, Michalak T, Mondelli MU, Pawlotsky JM, Pollicino T, Prati D, Puoti M, Samuel D, Shouval D, Smedile A, Squadrito G, Trépo C, Zoulim F. et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol 2008;49:652-7.

6. Mine H, Emura H, Miyamoto M, Tomono T, Minegishi K, Murokawa H, Yamanaka R, Yoshikawa A, Nishioka K; Japanese Red Cross NAT Research Group. High throughput screening of 16 million serologically negative blood donors for hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. J Virol Methods 2003;112:145-51.

7. Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K. Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. Transfusion 2007;47:1197-205.

8. Pharmaceutical and Food Safety Bureau, Blood and Blood Products Division. Guidelines for lookback study for blood products. Tokyo, Japan: Japanese Ministry of Health, Labour and Welfare; 2005. p. 2-26.

9. Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, Pappalardo B, Kleinman SH; NHLBI-REDS NAT Study Group. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005;45:254-64.

10. Satake M. Infectious risks associated with the transfusion of blood components and pathogen inactivation in Japan. Int J Hematol 2004;80:306-10.

11. Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K; Japanese Red Cross NAT Screening Research Group. HBV NAT positive [corrected] blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang 2005;88:77-86.

12. Kosaka Y, Takase K, Kojima M, Shimizu M, Inoue K, Yoshiba M, Tanaka S, Akahane Y, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. Fulminant hepatitis B: induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. Gastroenterology 1991;100:1087-94.

13. Sato S, Suzuki K, Akahane Y, Akamatsu K, Akiyama K, Yunomura K, Tsuda F, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. Ann Intern Med 1995;122:241-8.

14. Okamoto H, Yotsumoto S, Akahane Y, Yamanaka T, Miyazaki Y, Sugai Y, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M. Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. J Virol 1990;64:1298-303.

15. Kurosaki M, Enomoto N, Asahina Y, Sakuma I, Ikeda T, Tozuka S, Izumi N, Marumo F, Sato C. Mutations in the core promoter region of hepatitis B virus in patients with chronic hepatitis B. J Med Virol 1996;49:115-23.

16. Hoofnagle JH, Seeff LB, Bales ZB, Zimmerman HJ. Type B hepatitis after transfusion with blood containing antibody to hepatitis B core antigen. N Engl J Med 1978;298:1379-83.

17. Iizuka H, Ohmura K, Ishijima A, Satoh K, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. Correlation between anti-HBc titers and HBV DNA in blood units without detectable HBsAg. Vox Sang 1992;63:107-11.

18. Japanese Red Cross Non-A, Non-B Hepatitis Research Group. Effect of screening for hepatitis C virus antibody and hepatitis B virus core antibody on incidence of post-transfusion hepatitis. Lancet 1991;338:1040-1.

19. Nozawa Y, Ohto H. An autopsy case of post-transfusion fulminant hepatitis B due to screened blood for anti-hepatitis B core. Kanzo 1993;34:433-6.

20. Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti ML, Smaill F. Pathogen inactivation: making decisions about new technologies. Report of a consensus conference. Transfusion 2007;47:2338-47.

21. McCullough J. Pathogen inactivation: a new paradigm for preventing transfusion-transmitted infections. Am J Clin Pathol 2007;128:945-55.

22. Allain JP, Hewitt PE, Tedder RS, Williamson LM. Evidence that anti-HBc but not HBV DNA testing may prevent some HBV transmission by transfusion. Br J Haematol 1999;107: 186-95.

23. Grob P, Jilg W, Bornhak H, Gerken G, Gerlich W, Günther S, Hess G, Hüdig H, Kitchen A, Margolis H, Michel G, Trepo C, Will H, Zanetti A, Mushahwar I. Serological pattern "anti-HBc alone": report on a workshop. J Med Virol 2000; 62:450-5.

24. Gerlich WH, Wagner FF, Chudy M, Harritshoj LH, Lattermann A, Wienzek S, Glebe D, Saniewski M, Schüttler CG, Wend UC, Willems WR, Bauerfeind U, Jork C, Bein G, Platz P, Ullum H, Dickmeiss E. HBsAg non-reactive HBV infection in blood donors: transmission and pathogenicity. J Med Virol 2007;79:S32-S36.

25. Dreier J, Kröger M, Diekmann J, Götting C, Kleesiek K. Intermittent HBV viremia in an anti-HBc and anti-HBs-positive blood donor. Transfus Med 2005;15:65-6.

26. Stramer SL, Zou S, Notari EP, Foster GA, Krysztof DE, Musavi F, Dodd RY. Blood donation screening for hepatitis B virus markers in the era of nucleic acid testing: are all tests of value? Transfusion 2012;52:440-6.

27. Kino S, Tomoda Y, Itoh Y, Karasaki H, Kasai S. Implementation of pre- and post-transfusion viral marker tests at Asahikawa medical college hospital. Jpn J Transfus Cell Ther 2009;55:21-8.

28. Hollinger FB, Dodd RY. Hepatitis B virus traceback and lookback: factors to consider. Transfusion 2009;49:176-84.

29. Yang MH, Li L, Hung YS, Hung CS, Allain JP, Lin KS, Tsai SJ. The efficacy of individual-donation and minipool testing to detect low-level hepatitis B virus DNA in Taiwan. Transfusion 2010;50:65-74.

30. Weber B, Melchior W, Gehrke R, Doerr HW, Berger A, Rabenau H. Hepatitis B virus markers in anti-HBc only positive individuals. J Med Virol 2001;64:312-9.

31. Bréchot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? Hepatology 2001;34:194-203.

32. Alhababi F, Sallam TA, Tong CY. The significance of "anti-HBc only" in the clinical virology laboratory. J Clin Virol 2003;27:162-9.

33. Levicnik-Stezinar S, Rahne-Potokar U, Candotti D, Lelie N, Allain JP. Anti-HBs positive occult hepatitis B virus carrier blood infectious in two transfusion recipients. J Hepatol 2008;48:1022-5.

34. Inaba S, Ito A, Miyata Y, Ishii H, Kajimoto S, Tanaka M, Maruta A, Saito S, Yugi H, Hino M, Tadokoro K. Individual nucleic amplification technology does not prevent all hepatitis B virus transmission by blood transfusion. Transfusion 2006;46:2028-9.

35. Candotti D, Allain JP. Transfusion-transmitted hepatitis B virus infection. J Hepatol 2009;51:798-809.

36. Liu CJ, Lo SC, Kao JH, Tseng PT, Lai MY, Ni YH, Yeh SH, Chen PJ, Chen DS. Transmission of occult hepatitis B virus by transfusion to adult and pediatric recipients in Taiwan. J Hepatol 2006;44:39-46. ▣

B型肝炎
——最新治療コンセンサス2012

企画 **溝上雅史** 国立国際医療研究センター肝炎・免疫研究センター

CONTENTS

http://www.ishiyaku.co.jp/

■サイドメモ目次

献血者におけるHBV感染状況

Prevalence of HBV in volunteer blood donors in Japan

内田 茂治
Shigeharu UCHIDA
日本赤十字社血液事業本部中央血液研究所感染症解析部

◎献血者における HBs 抗原陽性率は，陽性通知の効果もあって年々低下してきている．年代別では 50 歳代をピークとして高齢者側で陽性率が高く，若年者側での陽性率は低い．とくに 1985 年 6 月から開始された，公費による "B 型肝炎の母子感染防止対策事業" 開始以降の出生者で陽性率の低下は顕著である．地域別では従来から報告されている北海道・九州の陽性率が高く，ついで中四国，近畿，東北，関東甲信越・東海北陸の順である．HBs 抗原陽性者の約 9 割は B 型肝炎ウイルス(HBV)キャリアと考えられ，残りの約 1 割は新規感染者と考えられた．HBV キャリアでは日本で多いといわれている遺伝子型 C と遺伝子型 B で大半を占めるが，新規感染者では遺伝子型 A が約 2 割を占め，外国型 HBV が性感染症として国内で広がっている．さらなる感染の拡大も危惧されており，HB ワクチン接種などの水平感染防止対策の検討が必要であると思われる．

Key word：献血者，HBs抗原陽性率，HBVキャリア，HBV新規感染，HBV遺伝子型

B 型肝炎はかつて日本の国民病といわれていたが，1985 年 6 月から全国の医療機関で開始された公費負担による "B 型肝炎母子感染防止対策事業" の成果により，それ以降の出生児には B 型肝炎ウイルス(HBV)キャリアが激減した[1,2]．また，成人における B 型急性肝炎は一過性の経過で治癒すると考えられてきたため，HBV による慢性肝疾患は将来きわめてまれになると考えられてきた．しかし近年，欧米型の B 型急性肝炎が性感染症として国内で急速に拡大し，しかも感染者の約 10%が慢性化するといわれているため，universal vaccination などのあらたな対策の必要性が議論されてきている[3]．

本稿では献血者における HBV 感染の現状を概説したい．

献血者における HBs抗原・HBc抗体陽性率

1965 年に Blumberg らによって，オーストラリア原住民の血清から血清蛋白の亜型とは異なる性質をもった抗原が発見された[4]．1968 年にこの抗原が血清肝炎と密接な関係にあることが報告され[5]，1970 年には HBV 粒子(Dane 粒子)が発見された[6]．日本では献血者の HBs 抗原検査が 1972 年 1 月から全国の血液センターで導入された．

明確なデータが存在する 1985 年以降の HBs 抗原陽性率を図 1 に示す．HBs 抗原陽性献血者には当初から陽性通知を行っていたため，その陽性率は 1987 年の 1.30%から連続的に低下し，2007 年には 0.04%(献血者 494 万人，HBs 抗原陽性者 2,036 人)にまで減少している．2008 年に検査法が従来の逆受身赤血球凝集試験(reversed passive hemagglutination test：RPHA 法)から化学発光酵素免疫測定法(chemiluminescent enzyme immunoassay：CLEIA 法)へと変更になり，2008 年の陽性率は 0.12%(献血者 508 万人，HBs 抗原陽性者 6,172 人)と急増した．当初は検出感度が数百倍上がったためと考えられたが，吸収試験陰性，HBc 抗体陰性，および HBV-DNA 陰性の擬陽性が多数含まれていることが判明した．また，HBc 抗体検査は 1989 年に導入され，2 度の合否基準変更により 1998 年には陽性率が 2.20%にまで