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一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	American Society of Hematology 46th Annual Meeting 2004; 2259.	公表国 日本	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社)					

研究報告の概要

肝臓で HBV が再活性化している状態はリバースセロコンバージョン(RS)として知られており、造血幹細胞移植(HSCT)の稀な合併症として報告されている。しかし、RS の正確な発生頻度や RS 後の長期追跡結果については報告されていないことから、同種 HSCT 前に HBs 抗体を保有していた患者 14 名における HBV 血清マーカーについてレトロスペクティブに検討した。HBs 抗体価の低下は 14 例全例で観察された。14 例中 12 例において、HSCT10～38 ヶ月後に HBs 抗体価が防御値以下まで低下した。RS は HSCT12～51 ヶ月後に 7 例でみられた。5 例は再度 HB s 抗体が陽転化したが、2 例は一過性肝炎の緩解後も依然として HBV キャリアであった。その他 5 例では HBs 抗体の消失後でも HBV は再活性化しなかった。HBs 抗体の消失および RS の実際のリスクは、それぞれ 2 年後 75.0%、39.8%、5 年後 100.0%、70.0%と予測された。HSCT 後の RS による肝炎は HBV に対するレシピエント由来の免疫が消失した後にドナーの免疫が再構築されることにより引き起こされるのであろう。RS は頻度の高い晩期合併症であり、HBs 抗体が徐々に低下していく経過を注意深く観察することで予測可能である。ドナーおよび/またはレシピエントに対するワクチン接種は、HBV 再活性化に対する予防療法となるであろう。

報告企業の意見	今後の対応
同種造血幹細胞移植（HSCT）前に HBs 抗体を保有していた患者 14 名における HBV 血清マーカーについてレトロスペクティブに検討したところ、肝臓で HBV が再活性化しているリバースセロコンバージョン（RS）となる可能性が示されたとの報告である。	HSCT 及び臓器移植を行った献血者については、問診で除外しており、また、スクリーニングにおいても、HB s Ag 及び NAT により HBV の感染リスクを排除している。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」

照射解凍赤血球濃厚液「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD 等の伝播のリスク

[2259] Progressive Disappearance of Anti-HBs and Reverse Seroconversion after allo-HSCT in Patients with Previous HBV Infection. Session Type: Poster Session 472-II

3

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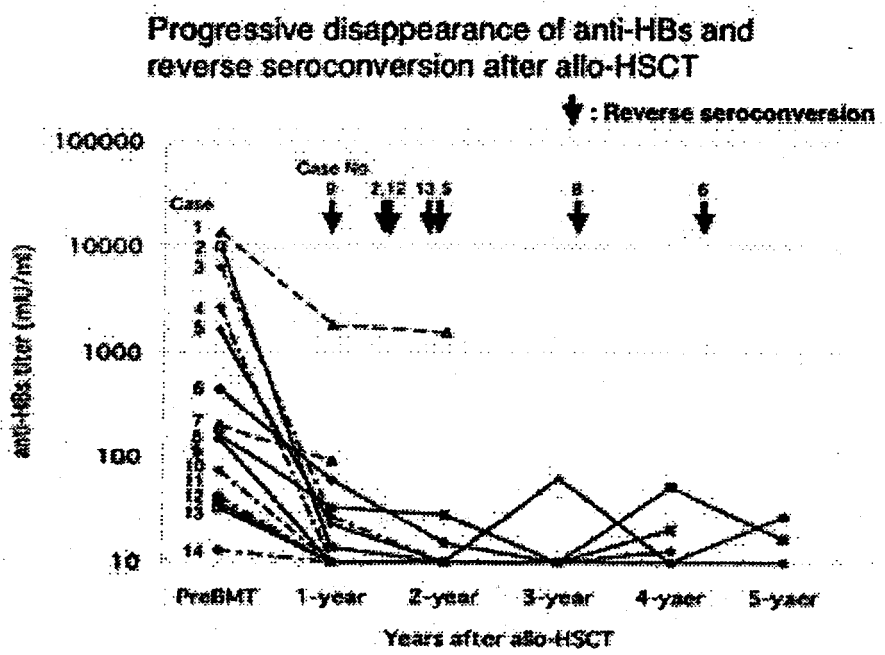
Development of anti-hepatitis B surface antigen antibody (anti-HBs) and clearance of hepatitis B virus (HBV) from serum usually indicate resolution of hepatitis in patients infected with HBV. However, most patients in whom HBV has been eliminated from the serum still have HBV DNA in the liver that is detectable by using polymerase chain reaction. Reactivation of this dormant HBV in the liver is known as reverse seroconversion (RS), which was reported to be a rare complication of hematopoietic stem cell transplantation (HSCT). However, the precise frequency of RS and results of long-term follow-up after RS have not yet been reported. We retrospectively studied HBV serological markers in fourteen patients with anti-HBs before allogeneic HSCT who were followed up for more than 1 year. Patients' characteristics are as follows: median age at time of HSCT, 35 years (range, 22-52 years); M:F ratio, 9:5; hematological disorders: CML 5, ALL 4, MDS 3, SAA 2. No patients had prior history of vaccination or HBV-specific immunoglobulin usage. All donors were negative for hepatitis B surface antigen (HBsAg), and four donors were confirmed to be negative for anti-HBs. Only one case (case 1) had relapse of hematological malignancy during the follow-up period. RS is defined as disappearance of anti-HBs and appearance of HBsAg, HBV-DNA, and clinical hepatitis. The follow-up period varied from 15 to 92 months (median, 48 months). The actual risk of disappearance of anti-HBs and RS were calculated using the Kaplan-Meier method. Progressive decreases in anti-HBs titer were observed in all 14 cases. In 12 of the 14 cases, anti-HBs titer had decreased to under the protective value (<10.0 mIU/ml) at 10 to 38 months (median, 13 months) after HSCT. RS occurred in 7 cases at 12 to 51 months (median, 20 months) after HSCT. Onset and severity of hepatitis were not correlated. Although re-seroconversion occurred in 5 cases, the other 2 cases remained in HBV carrier status after resolution of transient hepatitis. In the other 5 cases, reactivation of HBV did not occurre even after disappearance of anti-HBs. In case with RS, disappearances of anti-HBs occurred up to 18 months before the occurrence of RS. The actual risks of disappearance of anti-HBs and RS were estimated to be 75.0% and 39.8% at 2 years and 100.0% and 70.0% at 5 years, respectively. Progressive disappearance of anti-HBs would be an inevitable phenomenon accompanied by progressive loss of recipient-derived B cells. In our study, RS after allo-HSCT was not such a rare event. RS hepatitis after allo-HSCT would be caused by reconstructed naive donor immunity after loss of recipient-derived immunity against HBV. In conclusion, RS is a late-onset complication with high frequency that can be predicted by careful monitoring of progressive disappearance of anti-HBs. Pre-transplant vaccination of the donor and/or vaccination for recipients depending on the decrease in anti-HBs titer would be prophylactic for reactivation of HBV.

3



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Sunday, December 5, 2004, 06:00 PM

Poster Session: Late Effects, Chronic GVHD, and Second Cancers after Transplantation (6:00 PM-7:30 PM)

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識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2005.1.28	該当なし	
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	Vox Sang 2005; 88(1): 10-16.	公表国 日本	
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研究報告の概要

NAT 陽性ドナーは血清学的に陽性であるドナーとは感染の背景が異なると考えられる。今回、HBV、HCV が NAT 陽性となったドナーにおいてこの背景の特徴を明らかにする目的で調査を行った。抗体陰性ドナーに対し NAT を実施したところ、HBV DNA 陽性 328 名、HCV DNA 陽性 44 名が検出された。この結果は年齢、性別、HBV、HCV の genotype に関して特性づけられた。HBV NAT 陽性のドナーは大部分が若年者で、特に 10 代の少女が多かった。日本では、genotype C、genotype B が以前は優勢であったが、最近では genotype A が増加しており、genotype H も最近検出された。HBV NAT 陽性のドナーでは、genotype A 保有率が入院患者（1.7〜2%）に比較して高かった（12.2%）。HCV NAT 陽性のドナーも若年者が多かったが、主に 20 代の男性であった。HCV NAT 陽性のドナーにおける genotype 1b：2a あるいは 1b：2b の割合は、日本の入院患者のそれとは異なっていた。米国で優勢である genotype 1a は見つからなかった。NAT により検出されたハイリスクドナーは HBV、HCV 双方とも主に若年者で、入院患者とは異なる genotype の分布を有する。HBV の稀な genotype H が日本で初めて発見された。本調査結果は HBV、HCV が現在広がっていることを反映している。

使用上の注意記載状況・その他参考事項等

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報告企業の意見	今後の対応
日本の献血者において NAT により検出されたハイリスクドナーは HBV、HCV 双方とも主に若年者で、入院患者とは異なる genotype の分布を有する。HBV の稀な genotype H が日本で初めて発見された。本調査結果は若年層に HBV、HCV が現在広がっていることを反映しているとの報告である。	日本赤十字社は、平成 16 年 8 月 28 日より核酸増幅検査の感度を上昇させるためプールサイズを 50 から 20 へ変更した。また、HBV/HCV 感染の新たな伝播ルート等について、今後とも情報の収集に努める。

Vox Sanguinis (2005) 88, 10–16

ORIGINAL PAPER

Epidemiology of blood donors in Japan, positive for hepatitis B virus and hepatitis C virus by nucleic acid amplification testing

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Vox Sanguinis

Background and Objectives The Japanese Red Cross screens seronegative blood donors by nucleic acid amplification testing (NAT) for hepatitis B, hepatitis C and human immunodeficiency virus-1 markers. NAT-positive donors thus identified seemed to have a different infectious background from serologically positive donors. The purpose of our study was to characterize this background in the hepatitis B virus (HBV) and hepatitis C virus (HCV) NAT-positive donors.

Materials and Methods Some 328 HBV DNA-positive and 44 HCV RNA-positive donors were detected by NAT testing of seronegative blood donors. These were characterized regarding age, gender and genotype of HBV and HCV.

Results Those who were HBV NAT-positive were mainly young, in particular teenage girls. In Japan, genotypes C and B have previously been dominant, but recently genotype A has increased, and genotype H was recently detected. In HBV NAT-positive donors, the rate of genotype A was high (12·2%) compared with patients in hospital (1·7–2%). Donors who were HCV NAT-positive were also young, but mostly men in their twenties. The ratio of genotype 1b to 2a or 1b to 2b in HCV NAT-positive donors differed from that of hospitalized patients in Japan. We did not find genotype 1a, which is dominant in the USA.

Conclusions The high-risk donors detected by NAT were mainly young, with a different distribution of genotypes from that of hospitalized patients, regarding both HBV and HCV. The rare HBV genotype H has been found for the first time in Japan. The findings reflect the present spread of hepatitis viruses B and C.

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Key words: age and gender distribution, blood donors, epidemiology, genotypes of HBV DNA, genotypes of HCV RNA, nucleic acid amplification testing (NAT).

Introduction

Many countries perform routine nucleic acid amplification testing (NAT) for hepatitis C virus (HCV) and human immunodeficiency virus-1 (HIV-1) in blood donors. In Japan the endemicity of hepatitis B virus (HBV) makes HBV NAT an effective screening measure for using to prevent transfusion-associated hepatitis B. For that reason, Japan implemented nationwide HBV DNA screening for blood transfusion. We have already reported the efficacy of the HBV minipool NAT [1–5].

The Japanese Red Cross (JRC) carries out NAT screening for HCV, HBV and HIV-1 by using multiplex (MPX) reagents on serologically negative blood units from unpaid donations.

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HBV has been classified into genotypes A-G, and recently H [6]. HCV is also classified into several genotypes. These genotypes of HBV and HCV show a distinctive geographical distribution and a relevance to clinical severity [7–9]. They are important in epidemiological studies, in the analysis of modes of infection, and for medical treatment.

In this report, we have characterized NAT-positive donors regarding gender, age and genotypes of HBV and HCV.

Materials and methods

Minipool NAT and serological tests

On 1 July 1999, the JRC started nationwide NAT screening on pools of 500 serologically negative donations. From 1 February 2000, the pool size was reduced to 50. By 31 March 2003, 17 314 486 units had been tested this way on 50-sample pools with an input volume of 0·2 ml of each pool. Mine *et al.* have already reported the NAT screening system in Japan [1]. In brief, the eligibility of all volunteer blood donors in the JRC blood centres is determined by a questionnaire used throughout Japan. The blood samples are screened by using serological tests [1,3,5,10]. Serologically positive or alanine aminotransferase (ALT)-elevated (> 60 IU/L) samples are excluded from NAT screening.

Serological screening for HBV is carried out by using reverse passive haemagglutination (RPHA) for hepatitis B surface antigen (HBsAg), haemagglutination inhibition (HI) for antibody to HBV core antigen, and passive haemagglutination (PHA) for antibody to HBsAg. The JRC prepares the reagents, and we have previously described the criteria for classification as serologically positive [1,5,10]. In Japan, serological screening for HCV is carried out by using PHA (Abbott Laboratories, Abbott Japan, Co., LTD. Minato-Ku, Tokyo, Japan) or particle agglutination (PA) (Ortho-Clinical Diagnostics, K.K. Kahtoh-Ku, Tokyo, Japan). Serologically positive titres are defined by the manufacturers: a titre of $\geq 2 \times 10^5$ is defined as positive by PHA, and a titre of $\geq 2 \times 10^4$ is positive by PA.

Determination of the genotypes of HBV and HCV

The genotypes of HBV and precore or core promoter mutations were detected and characterized according to Okamoto *et al.* [11,12]. To sequence the HBV DNA, the following primers from the HBsAg region were used: S1-1 (sense: 5′-TCGTGTTACAGGCGGGGTTT-3′; nucleotides 192–211), S1-2 (antisense: 5′-CGAACCACTGAACAAATGGC-3′; nucleotides 685–704), S2-1 (nested sense: 5′-CAAGGTATGTTGCCCG-TTTG-3′; nucleotides 455–474) and S2-2 (nested antisense: 5′-GGCACTAGTAAACTGAGCCA-3′; nucleotides 668–687). The following primers from the hepatitis B virus core (HBc) region were used: HB104 (sense: 5′-AGACCACCAAAT-GCCCCTATC-3′; nucleotides 2297–2317), HB105 (antisense: 5′-CCCACCTTATGAGTCCAAGG-3′; nucleotides 2463–2482), HB106 (nested sense: 5′-CCCCTATCYTATCMACACTTCCG-3′; nucleotides 2310–2332), HB107 (nested antisense: 5′-GAGATTGAGATCTTCTGCGAC-3′; nucleotides 2421–2441). The following primers from the precore region were used: PC1-1 (sense: 5′-CATAAGAGGACTCTTGGACT-3′; nucleotides 1653–1672), PC1-2 (antisense: 5′-AAAGAATTCAGAAGGCAAA-AAAGA-3′; nucleotides 1949–1972), PC2-1 (nested sense: 5′-AATGTCAACGACCGACCTTG-3′; nucleotides 1679–1698) and PC2-2 (nested antisense: 5′-TCCACAGAAGCTCCGAATTC-3′; nucleotides 1922–1941). To sequence the HBV DNA of genotype A of 1600 nucleotides, HB104 and S1-2 were used for the first polymerase chain reaction (PCR), and HB106 and S2-2 were used for the nested PCR. To determine the full sequence of the HBV DNA of genotype H of 3215 nucleotides, three regions (a, b and c) were PCR amplified separately. The 'a' region was the same as the above genotype A of 1600 nucleotides. The 'b' region was amplified by using the primers S2-1 and #038 for the first PCR, and #066 and #039 for the nested PCR. The 'c' region was amplified by using the primers PC1 and #060 for the first PCR, and #012 and #062 for the nested PCR. The primers of # are as follows. #038 (antisense: 5′-AAAGTTGCATGGTGCTGGTG-3′; nucleotides 1804–1823), #066 (nested sense: 5′-TATGTTGCCCGTTTGTC-CTC-3′; nucleotides 460–479), #039 (nested antisense: 5′-ATGGTGCTGGTGAACAGACC-3′; nucleotides 1796–1815), #060 (antisense: 5′-GATTGAGATCTTCTGCGACG-3′; nucleotides 2414–2433), #012 (nested sense 5′-AATGTCAACGAC-CGACCTTG-3′; nucleotides 1679–1698) and #062 (nested antisense 5′-CTTCGTCTGCGAGGCGAGGG-3′; nucleotides 2381–2400). Sequencing was carried out directly by using the BigDye Terminator Cycle Sequencing Kit and the ABI PRISM 3100 Genetic Analyser (PE Applied Biosystems, Foster City, CA). To analyse the sequences, SEQUENCHER MAC Version 4·1 (Hitachi Software Engineering, Tokyo, Japan) or GENETYX MAC Version 9·0 (Software Development, Tokyo, Japan) were used.

The genotypes of HCV were determined by using the HCV GENOTYPE Primers Kit (Institute Immunology, Tokyo, Japan) that was based on the methods of Okamoto *et al.* [13].

Results

Characterization of HBV NAT-positive and HCV NAT-positive donors

The age and gender of the 328 HBV NAT-positive donors are shown in Table 1. Most of the NAT-positive men were in their twenties and thirties; most women in their twenties. However, when the figures were expressed per 100 000, teenage girls had the highest rate of HBV NAT-positivity, at 3·35 donors per 100 000 (Fig. 1a). Figure 1(b) shows the serological prevalence rate of HBsAg per 100 000 first-time donors in 2000. Contrary to the results of the NAT-positive

Table 1 The actual number of nucleic acid amplification testing (NAT)-positive donors by age and gender

Age	HBV NAT-positive donors		HCV NAT-positive donors	
	Male	Female	Male	Female
16–19	15	33	2	3
20–29	90	70	16	5
30–39	55	14	3	3
40–49	9	9	4	2
50–59	16	6	2	0
60–69	8	3	3	1
Total	193	135	30	14

HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 2 The ratio of first-time donors to repeat donors in nucleic acid amplification testing (NAT)-positive donors

	HBV NAT-positive donors		HCV NAT-positive donors	
	Number	%	Number	%
First-time donors[a]	66	20·1	19	43·2
Repeat donors	262	79·9	25	56·8
Total	328	100·0	44	100·0

[a]The ratio of first-time donors to total donors is ≈ 10% in normal Japanese donors.

donors, HBsAg positivity was found to increase gradually with age.

Table 1 shows the age and gender of the 44 HCV NAT-positive donors, while Fig. 2(a) provides the number corrected per 100 000 donors. The high rate of positives is noteworthy in men in their sixties, in addition to men in their twenties. Figure 2(b) shows the rate of anti-HCV-positives per 100 000 first-time donors in the year 2000. Like HBsAg, the serological positivity for HCV increases with age.

Table 2 shows the ratio of first-time to repeat NAT-positive donors. For HCV, 19 NAT-positives (43·2%), and for HBV 66 (20·1%) first-time donors were identified. The rate of NAT-positive donors for both markers exceeded the overall rate of

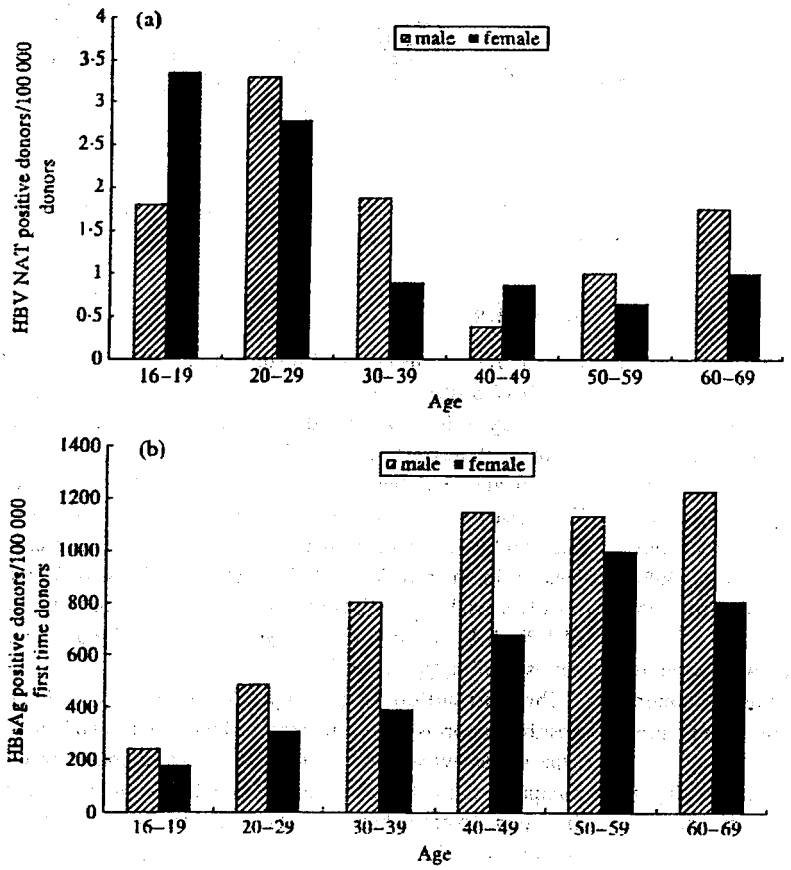

Fig. 1 Age and gender of hepatitis B virus (HBV) donors positive by nucleic acid amplification testing (NAT) and serologically positive donors. Out of 328 HBV NAT-positive donors, 193 were male and 135 were female. (a) The vertical axis shows HBV NAT-positives/100 000 donors and the horizontal axis the age. (b) The vertical axis shows the hepatitis B surface antigen (HBsAg) (serological) positives/100 000 first-time donors and the horizontal axis the age. This figure shows the present prevalence of HBV infection in Japan (data from the year 2000).

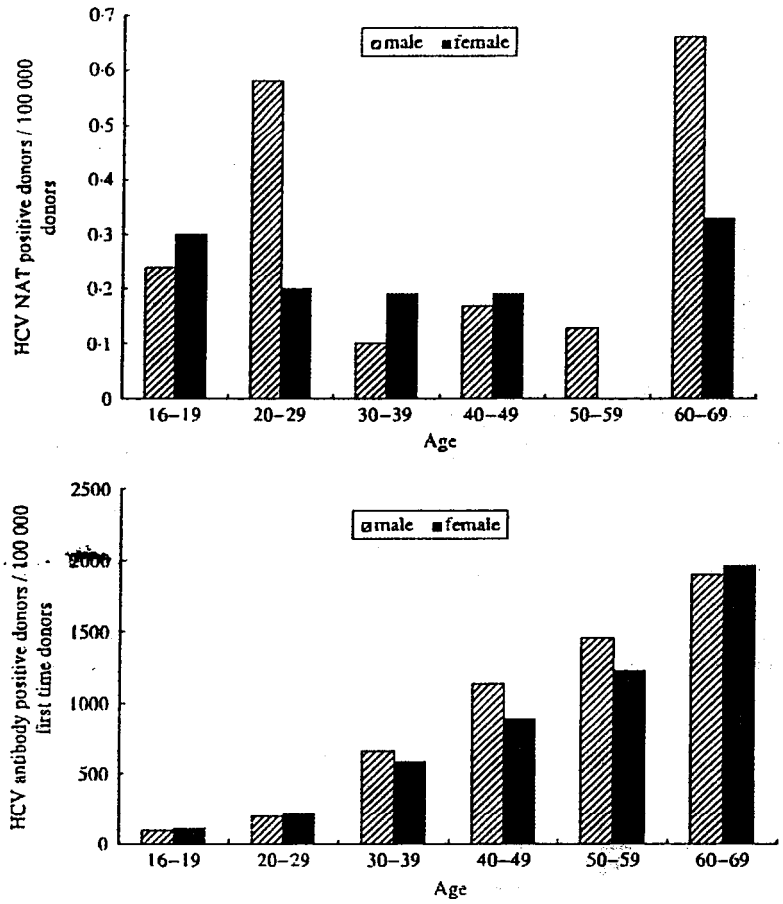

Fig. 2 Age and gender of hepatitis C virus (HCV) donors positive by nucleic acid amplification testing (NAT) and serologically positive donors. Out of 44 HCV NAT-positive donors, 30 were male and 14 were female. (a) The vertical axis shows HCV NAT-positives/100 000 donors, and the horizontal axis the age. (b) The vertical axis shows the HCV antibody (serological) positives/100 000 first-time donors and the horizontal axis the age. This figure shows the present prevalence of HCV antibody in Japan (data from the year 2000). The present HCV infection (HCV RNA-positive) rate might be 50% of HCV antibody-positive donors.

first-time donors in Japan (10%). Informed consent to follow-up the donors was more readily obtained (61·6%) from the HBV group than from the HCV group (52·3%).

Distribution of HBV and HCV genotypes in NAT-positive donors

The distribution of HBV genotypes in NAT-positive donors is shown in Table 3, together with published data relating to the distribution of genotypes seen in populations of patients in Japan. The relatively high incidence of genotype A infection is of particular note. Twenty-six of the genotype A cases were sequenced using 1600 bp (nucleotides 2310–3221/1–668). Twenty-five of these showed the US genotype A, with only one showing the Asian genotype A [16].

Precore and/or core promoter mutations were seen in genotypes A (5%), B (19%) and C (19%) but not in donors with genotype D or H. It is noteworthy that there were no precore mutations seen in genotype A donors.

Genotype 1b is dominant in HCV patients in Japan [9,17,18]. In NAT-positive donors, however, genotypes 2a and 2b were seen in 47% and 22% of cases (Table 4). Genotype 1a, the dominant genotype in the USA, was not found in the NAT-positive donors.

Discussion

This report contains information on a large series of HBV and HCV NAT-positive donors. The data indicate that the epidemiology of NAT-positive donors differs from that seen in donors with positive serology for these viruses.

There are a number of differences between HBV and HCV NAT-positive donors. First, HBV NAT positivity was predominantly found in teenage girls and in both males and females in their twenties. HCV NAT positivity was found mainly in males in their twenties and sixties. Second, 20·1% of HBV NAT-positive donors were first-time donors. The corresponding figure for HCV NAT was 43·2%.

The distribution of genotypes identified in the NAT-positive donors is also of interest. An increased level of genotype A was evident in the HBV NAT-positive donors when compared to the frequency of this genotype seen in patients in Japan. Overall, however, the frequency of genotype A has increased in both groups. Twenty-six of the genotype A

Table 3 Distribution of hepatitis B virus (HBV) genotypes among HBV nucleic acid amplification testing (NAT)-positive donors (with publication references)

HBV NAT-positive donors									
	Number			Mutation			Patients[d]	Patients[e]	Donors[f]
Genotype	*n* = 328	(%)	Wild-type *n* = 273	Precore[a] *n* = 15	Core-promotor[b] *n* = 27	Mix[c] *n* = 13	Japan (%) *n* = 1077	Japan (%) *n* = 720	USA (%) n = 418
A	40	12·2	38	0	2	0	2	1·7	34·2
B	36	11·0	29	6	0	1	9	12·2	7·4
C	246	75·0	200	9	25	12	88	84·7	14·1
D	5	1·5	5	0	0	0	0·2	0·4	19·9[g]
H	1	0·3	1	0	0	0	0	0	0
F	0	0	0	0	0	0	0·2	0	1·0
Mix or undetermined	0	0	0	0	0	0	0·6	1·0	23·4

[a]Mutation from G to A at nucleotide 1896 of the precore region (wild-type: G; mutation: A) [11].
[b]Mutation from A to T at nucleotide 1762 and/or from G to A at nucleotide 1764 of the core promoter region (wild-type: A; mutation: T at nucleotide 1762, and wild-type: G; mutation: A at nucleotide 1764) [12].
[c]Both precore and core promotor mutations.
[d]Kobayashi *et al.* [8].
[e]Orito *et al.* [14].
[f]Moriya *et al.* [15].
[g]Genotype D or E.

Table 4 Distribution of hepatitis C virus (HCV) genotypes among HCV nucleic acid amplification testing (NAT)-positive donors (with publication references)

HCV NAT-positive donors							
	Number		Patients[a]	Donors or patients[b]		Patients[c]	
Genotype	*n* = 44	(%)	Japan (%) *n* = 1380	Japan (%) *n* = 302	US (%) *n* = 105	Japan (%) *n* = 379	USA (%) *n* = 63
1a	0	0·0	0·6	0·3	52	2·6	39·7
1b	13	29·6	70·6	54	22	64·4	31·7
2a	21	47·7	16·0	31	5	16·9	6·3
2b	10	22·7	4·9	12	11	12·4	14·3
3a	0	0	0·1	0	10	1·3	3·2
Mix or undetermined	0	0	7·8	0	0	2·4	4·8

[a]Tanaka *et al.* [9], modified the representation as a percentage.
[b]Okamoto *et al.* [17].
[c]Ohno *et al.* [18], modified the representation as a percentage.

donors were sequenced using 1600 bp (nucleotides 2310–3221/1–668). Twenty-five of the 26 showed a US pattern of genotype A, with only one showing the normal Asian pattern of genotype A [16]. This suggests that whilst HBV is endemic in Japan, a number of new HBV genotypes might have entered the country in recent years. A single case of genotype H was found; this is the first reported case from Japan. Interestingly, however, the picture seen in HCV NAT-positive donors was different. In particular, no cases of genotype 1a, the dominant form seen in the USA, were detected in our NAT-positive donors.

Core promoter and precore promoter mutations were detected in ≈ 15–20% of HBV NAT-positive donors of genotypes B and C. Conversely, these mutations were seldom seen in donors with HBV genotypes A and D. No precore mutations were found in donors with genotype A [19]. The presence or absence of these mutations is of significance given the pattern of fulminant hepatitis in Japan. Most cases of fulminant

hepatitis B in Japan, where HBV genotypes B and C predominate, have been caused by mutant strains [20–24]. In Europe, North America and Africa, however, where genotypes A and D are common, fulminant hepatitis is usually associated with wild-type strains.

In conclusion, monitoring of the epidemiology and genotype distribution of NAT-positive donors in Japan has highlighted that this population differs significantly from that seen in donors with positive serology and also in Japanese patients. Surveillance of this group of donors may provide early evidence of changes in the overall pattern of these infections.

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