Table 2. Rate of HBsAg-positive first-time blood donors born from 1980 to 1991

Year of birth	Age									
	16	17	18	19	20	21	22	23	24	25
1980	0·399	0·390	0·312	0·303	0·245	0·330	0·274	0·243	0·288	0·208
1981	0·313	0·279	0·281	0·285	0·289	0·232	0·326	0·240	0·219	0·205
1982	0·223	0·210	0·209	0·203	0·186	0·238	0·215	0·190	0·163	0·185
1983	0·142	0·179	0·164	0·144	0·157	0·157	0·096	0·170	0·154	
1984	0·129	0·105	0·117	0·130	0·106	0·076	0·139	0·134		
1985	0·105	0·110	0·086	0·126	0·078	0·070	0·126			
1986	0·055	0·035	0·056	0·067	0·061	0·098				
1987	0·040	0·044	0·049	0·058	0·071					
1988	0·044	0·020	0·038	0·041						
1989	0·017	0·024	0·044							
1990	0·041	0·036								
1991	0·018									

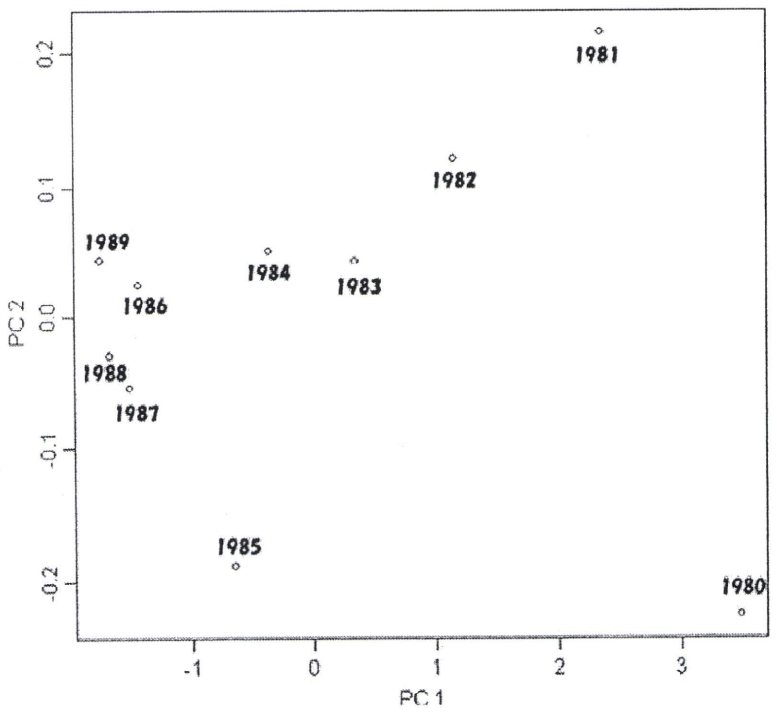

Fig. 2. Two-dimensional display of results of PCA of rate of HBsAg-positive first-time blood donors. Calculation was carried out with the free software 'R' (http://www.r-project.org/) using data within the frame of the dotted line in Table 2 (16-, 17- and 18-year-old donors born from 1980 to 1989). PCA is a statistical method to compress the multi-dimensional space of data into small-number- dimensions-space data (Appendix). The relations of data compressed into two dimensions are easy to understand by making a glance of this figure. The horizontal and vertical axes of PC 1 and PC 2 are composed of many types of variables. Therefore, it is difficult to show them as definite indexes. However, if we would be forced to consider the meaning of these axes, we had better regard them as the difficulty of determining infection by age. The group in 1986–1989 is obviously different from the other three groups in 1980, 1985 and 1981–1984.

Donors born after the implementation of the vaccination programme from 1986 to 1989 comprised one group. Donors born before the implementation of the vaccination programme can be divided into three groups; donors born in the transitional period in 1985 comprised one group, those born during the period of decreasing rate of HBsAg-positive donors from 1981 to 1984 comprised another group and those born during

the period of decreasing but rather high rate of HBsAg-positive donors in 1980 comprised an other single group.

The statistical significance of changes in decreasing curve and rate of HBsAg-positive donors in 1986 were investigated by regression analysis using Equation (1) described in Materials and Methods section

When 'n' is 16 years old, Equation (1) can be written as

$$y_{16} = \alpha_{16} + \beta_{16}x_1 + \gamma_{16}x_2 + \delta_{16}D + \varepsilon_{16} \quad (2)$$

When the birth of year is between 1980 and 1985, Equation (2) can be rewritten as follows, because x_1 = (1980–1991 : 0·399, 0·313, 0·223, 0·142, 0·129, 0·105, 0·055,0·040, 0·044, 0·017, 0·041, 0·018), x_2 = (1980–1991:0, 0, 0, 0, 0, 0, 0·055, 0·040, 0·044, 0·017, 0·041, 0·018, because γ is the additional coefficient of slope after 1986), D = (1980–1991: 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1, 1, because δ is the coefficient (D) that shows the gap of HBsAg-positive rate around 1986).

$$y_{16} = \alpha_{16} + \beta_{16}x_1 + \varepsilon_{16} \quad (3)$$

When the birth of year is after 1986, Equation (2) can be rewritten as follows, because after 1986, x_1 is equal to x_2.

$$y_{16} = (\alpha_{16} + \delta_{16}) + (\beta_{16} + \gamma_{16})x_2 + \varepsilon_{16} \quad (4)$$

P-values of β_{16-19}, γ_{16-19}, δ_{16-19} are shown in Table 3. All the terms between 16 and 18 are significant ($P < 0·05$). That is, the rate of HBsAg-positive donors decreased significantly yearly after 1980 (β_{16-19}), and the trend of decreasing slope before 1985 was significantly different from that after 1986 (γ_{16-18}). The difference in infectious rate was significant at the border line between 1985 and 1986 (δ_{16-18}). These data show that HBsAg-positive rate has decreased significantly in donors born from 1980 to 1991 (β_{16-19} are negative) regardless of the hepatitis B vaccination programme. There was a significant decrease in HBsAg-positive rate in donors born before 1985 and after 1986 (δ_{16-18} are negative). This drop would result from the effect of the hepatitis B vaccination programme. The additional coefficient γ_{16-18} is positive means that after the significant drop, the decreasing curve of HBsAg-positive rate became flat, because there might be no room to decrease by vertical transmission except a few cases as intrauterine transmissions and inappropriate vaccinations. The remaining HBsAg-positive rate might be caused by horizontal transmission. A significant change was not observed for 19 years old [asterisk symbol (*) in Table 3], because the data of this age group were considered to be too small to obtain a significant difference statistically.

DISCUSSION

HBsAg-positive first-time blood donors consist of both horizontally and vertically infected donors. The minimum number of HBV-positive first-time blood donors with consistently horizontal infection from October 2006 to September 2007 was anticipated to be 94, on the basis of the sum of the numbers of HBsAg-negative and NAT-positive (68:90–22), and IgM-HBcAb-positive (26:61–35) donors, which was 4·4% (94/2133) of the total number of HBsAg-positive donors (2043) plus 90 HBsAg-negative and NAT-positive donors. The exact number of horizontally infected donors was obscure, making it difficult to determine the effectiveness of the Japanese vaccination programme for the prevention of mother-to-infant transmission of HBV. Moreover, the total number of HBsAg-positive donors was decreasing yearly before the start of the prevention programme. The number of HBsAg-positive first-time blood donors in several prefectures was actually too small to treat statistically in the investigation of the effectiveness of the Japanese

Table 3. Data obtained using Equation (1)

	Coefficient	Standard error	t-Value	P-value		Coefficient	Standard error	t-Value	P-value
α_{16}	0·429	0·025	17·375	1·2E−07	α_{17}	0·407	0·025	16·419	7·6E−07
β_{16}	−0·060	0·006	−9·482	1·3E−05	β_{17}	−0·056	0·006	−8·756	5·1E−05
γ_{16}	0·054	0·009	6·038	**3·1E−04**	γ_{17}	0·054	0·011	5·110	**1·4E−03**
δ_{16}	−0·336	0·066	−5·098	**0·001**	δ_{17}	−0·359	0·081	−4·454	**0·003**
α_{18}	0·362	0·009	39·749	1·7E−08	α_{19}	0·339	0·022	14·906	2·5E−05
β_{18}	−0·048	0·002	−20·394	9·0E−07	β_{19}	−0·040	0·006	−6·890	9·9E−04
γ_{18}	0·043	0·005	8·665	**1·3E−04**	γ_{19}	0·027	0·018	1·493	**0·196***
δ_{18}	−0·275	0·004	−7·130	**0·0004**	δ_{19}	−0·180	0·141	−1·278	**0·257***

*γ_{19} and δ_{19} are not significant ($P < 0·05$)

prevention programme (Chiyoda *et al.*, 2006; Uchida and Tadokoro, 2008). The situation was similar to the case of HCV. The rate of HCV-positive first-time blood donors in Japan has declined (data not shown). O'Brien *et al.* (2008) reported that they could not determine why the infection rates of HCV have decreased in Canada.

If the Japanese prevention programme succeeded completely, HBV infection would only be caused by horizontal transmission after 1986, and the trend of declining slope after 1986 would be different before 1985. However, in spite of the prevention programme, vertical transmission remained because of intrauterine transmissions and inappropriate vaccinations or problems of escape mutants. It was reported that 1·3% of infants became carriers immediately after birth before vaccination and another 2·1% became carriers during or immediately after the third vaccination because these infants were considered to be poor responders (Shiraki, 1994). Inui *et al.* (2007) reported that out of 27 patients who became infected, despite the immunoprophylaxis trials (selective vaccination and HBIG administration), 14 were infected by receiving an inappropriate Japanese vaccination programme, 11 were suspected to be infected by the intrauterine route and only 1 was infected by routes other than the mother-to-infant route. Therefore, a constant rate of vertically transmitted HBsAg-positive donors would remain in spite of universal or selective vaccination during infants. However, those infants who became infected despite the vaccination would be notified of the fact by a health centre or a hospital and should not donate blood or would be rejected to donate on the basis of their responses to a questionnaire. The problem might be the existence of donors infected by paternal or iatrogenic transmission routes, who were unaware of their being HBV carriers themselves or their parents. Some donors who were engaged in risk behaviour might visit a blood centre to donate and determine whether they were infected with viruses causing sexually transmitted diseases. In consideration of these factors, significant differences in the rate of HBV-positive donors were observed between before and after 1986.

The progressive decrease in the rate of HBV-positive donors from 1996, as shown in Fig. 1, might be due to the policy of the Ministry of Health and Welfare. To prevent an iatrogenic HBV infection, a vaccination enforcement regulation 'to use a disposable needle per person' was issued in September 1958 and the use of disposable syringes was permitted from September 1976. Afterwards, disposable needles and syringes were disseminated yearly and a memorandum 'to use a disposable syringe per person' was issued from the Ministry of Health and Welfare in January 1988.

According to the recommendation of the World Health Organization (WHO), many countries have implemented universal vaccination (World Health Organization, 1992) except UK (Hanè *et al.*, 2004) and Japan (Shiraki, 1994; Shiraki *et al.*, 1996; Inui *et al.*, 2007). Although there are many reports about the effectiveness of universal vaccination (Ni *et al.*, 2007; Gervais *et al.*, 2008; Mele *et al.*, 2008), reports about the comparison between the effectiveness of universal vaccination and that of selective vaccination are few. In Bulgaria, the period of selective vaccination of newborns to HBsAg-positive mothers was 1988– 1991, and that of the universal infant vaccination was thereafter (Hens *et al.*, 2008). Although they estimated the impact of vaccination using age–time-dependent incidence rates of hepatitis B, they did not show the superiority of universal vaccination to selective vaccination.

From the view points of cost–benefit and side effects, it should be considered which would be effective to implement, universal vaccination or selective vaccination with co-administration of HBIG to use the healthcare budget effectively. Although the side effect of HBV vaccine was estimated to be very low (Mikaeloff *et al.*, 2007), we cannot exclude the risk completely. Therefore, to continue the current Japanese strategy (selective vaccination) to control HBV infection or to implement universal vaccination is still open to discussion in Japan.

Although vertical transmission of HBV would be prevented sufficiently by the current selective vaccination, it might be necessary to prevent horizontal infection. The increase in horizontal HBV infection, especially HBV genotype A originated from United States or Western Europe, is apparent (Murokawa *et al.*, 2005). This might be supported by the finding that the HBV genotype A, which has been rare in Japan, was predominant among HBV–HIV dually infected Japanese men who had sex with other men (MSM). The sequences of genotype A spread by MSM were highly homologous to those of the strains isolated in the United States (Koibuchi *et al.*, 2001). In addition to genotype A, we have recently found genotype H in a Japanese HBsAg-negative and NAT-positive blood donor. The sequence of genotype H, which is prevalent only in the United States and Central America, was highly homologous to those of the strains isolated in Los Angeles (Ohnuma *et al.*, 2005).

HBV vaccination is not mandatory but recommended to workers engaged in medical services and to travellers who go to HBV endemic areas to reduce the horizontal infection. The implementation of universal vaccination as discussed above seems to be a solution; however, there is a problem of a 'waning-off' effect (Su *et al.*, 2008). The other solution is to immunize blood donors,

which was proposed in place of the implementation of NAT (Ringwald *et al.*, 2005). The vaccination of blood donors would reduce the risk of post-transfusion HBV infection. Although we do not know the effect of vaccination on occult HBV infection that is an important problem in the field of transfusion, it might also reduce the risk of occult HBV infection. In addition to reducing the risk, the vaccination of blood donors might be useful to produce HBIG because of the lack of HBsAb-positive plasma.

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APPENDIX

Principal component analysis (PCA) involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components.

Actually, it functions as eigenvector-based multivariate analyses. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible.

Accordingly, its operation can be thought of as revealing the internal structure of the data in a way which best explains the variance in the data. If a multivariate dataset is visualized as a set of coordinates in a high-dimensional data space (one axis per variable), PCA supplies the user with a lower-dimensional picture, a 'shadow' of this object when viewed from its (in some sense) most informative viewpoint.

Therefore, if we apply PCA to the obtained data, we will be able to observe several groups which will have the same or similar features. In addition, PCA can show the result visually, it is rather easy to understand the grouping intuitively.

Outline of calculation: The correlation matrix is calculated using the data within the frame of the dotted line in Table 2.

X_{16}	X_{17}	X_{18}
1	0·9866428	0·9855587
0·9866428	1	0·9843252
0·9855587	0·9843252	1

The maximum eigen value is $\lambda_1 = 2 \cdot 96978028$. Corresponding eigenvector is 0·5762051, 0·8111528 and 0·1000940, respectively. The first component is shown as follows:

$$Z = 0{\cdot}5762051(X_{16} - 0{\cdot}1880000) + 0{\cdot}8111528 \times (X_{17} - 0{\cdot}1698782) + 0{\cdot}1000940(X_{18} - 0{\cdot}1597273) = 0{\cdot}5762051X_{16} + 0{\cdot}8111528X_{17} + 0{\cdot}1000940X_{18} - 0{\cdot}2620628$$

The component loading of first principal component (correlation coefficient between original data and principal component score) is 0·9929772, 0·9963599 and 0·9955117, respectively.

Incidentally, correlation coefficient is shown as (eigenvector) × √(eigen value).

Then the change of Z is calculated according to 10 group of values (1980, 1981,. . ., 1988, 1989), principal component score (PC 1) is shown as follows:

3·71767980, 2·29772699, 1·42799966, 0·51159502, −0·65596423, −0·86558646, −1·47020213, −1·51833807, −1·65244844, −1·69703017.

This represents the total change as a change of one series.

Similarly, second eigen value is $\lambda_2 = 0 \cdot 02113356$. Corresponding eigenvector is 0·5781680, −0·3179823 and −0·7514047, respectively.

$$U = 0{\cdot}5781680(X_{16} - 0{\cdot}1880000) - 0{\cdot}3179823 \times (X_{17} - 0{\cdot}1698182) - 0{\cdot}7514047 \times (X_{18} - 0{\cdot}1597273) = 0{\cdot}5781680X_{16} - 0{\cdot}3179823X_{17} - 0{\cdot}751404X_{18} + 0{\cdot}0653234$$

Among the change of X_{16}, X_{17} and X_{18}, $(\lambda_2 + \lambda_2)/3$ is shown as 0·9969713 using the values of Z and U. This means that most information (99·7%) is converged in first and second principal component.

Such a method of analysis is called component analysis. Z and U are called first and second principal components, respectively. Fig. 2 is drawn by plotting the first principal component in X-axis and the second principal component in Y-axis.

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肝　炎

献血者におけるB型肝炎ウイルスと輸血後肝炎

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◆1．はじめに

B型肝炎はかつて日本の国民病といわれていたが，1986年から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」の効果により，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極めてまれになると考えられてきた．しかしながら，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などの新たな対策の必要性が議論されてきている．本稿では献血者におけるHBVの現状を輸血用血液に対するスクリーニング検査結果から考察し，併せて輸血による肝炎ウイルス伝播の実態を述べる．

◆2. 献血者におけるHBs抗原・HBc抗体陽性率

1968年に血清肝炎と密接な関係にある抗原が発見され，1970年にはHBV粒子（Dane粒子）が発見された．日本では献血者のHBs抗原検査が1972年に導入された．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％にまで減少している．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%にまで上昇したが，その後徐々に低下し，2003年の陽性通知開始以降急激に減少した．

一方，陽性通知による制約を受けない初回献血者のHBs抗原陽性率は，調査を開始した1995年から0.45～0.50%と横ばいであったが，2003年以降低下して2007年には0.21%まで減少してい

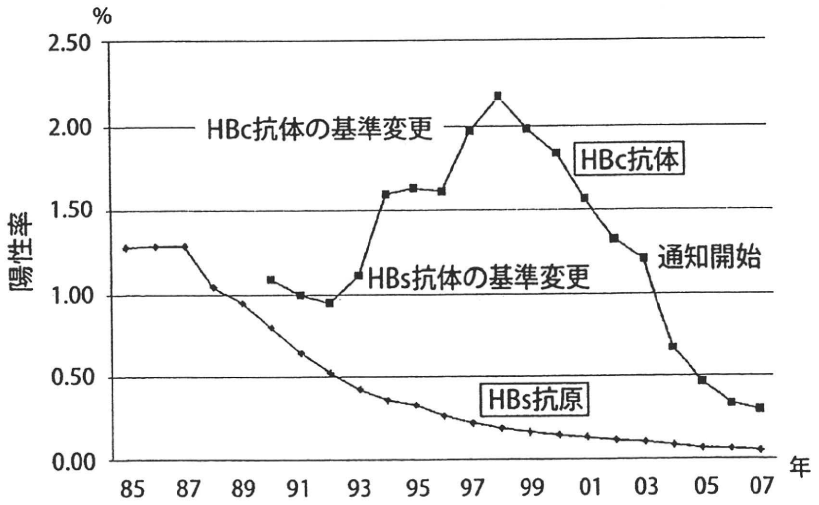

図1　献血者におけるHBs抗原・HBc抗体陽性率

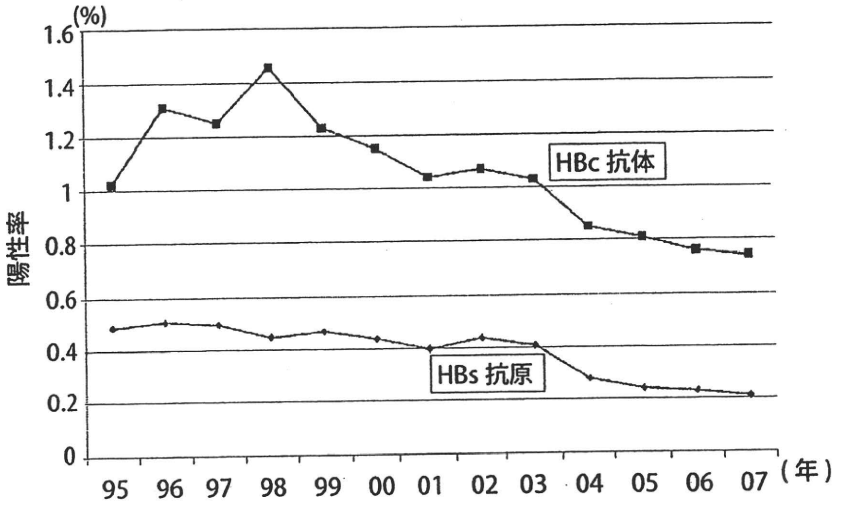

図 2　初回献血者における HBs 抗原・HBc 抗体陽性率

る (図 2). この初回献血者の HBs 抗原陽性率が我が国の献血者年代層 (16 ～ 64 歳) の陽性率を反映していると考えられる.

◆ 3. 献血者から見出される HBV の遺伝子型

HBV は現在 A ～ H の 8 つの遺伝子型に分類されており，我が国の HBV キャリアは遺伝子型 C が 84.7% を占め，次いで遺伝子型 B 12.2%，遺伝子型 A 1.7%，遺伝子型 D 0.4% で，その他の遺伝子型は検出されなかったと報告されている [3]. 他方，献血者から見出される HBV の遺伝子型については，2006 年 10 月から 2007 年 9 月までの 1 年間に HBs 抗原検査で 1998 例 (献血者数 4,959,541 人) の陽性例が検出され，遺伝子型が解析できた 1887 例 (男性 1372 人 : 女性 515 人) の集計を行った. その結果は遺伝子型 C 1181 例 (62.6%)，遺伝子型 B 581 例 (30.8%)，遺伝子型 A 106 例 (5.6%)，遺伝子型 D 15 例 (0.8%)，遺伝子型 E 2 例 (0.1%)，遺伝子型 F 2 例 (0.1%) であった. これらの HBs 抗原陽性例には HBV キャリアと新規感染者とが含まれているが，HBV キャリア例の報告に比べ遺伝子型 A と遺伝子型 B の比率が高くなっており，そのほか国内では非常に稀な遺伝子型 E や遺伝子型 F も見出されている. また，輸血用血液に対するスクリーニング核酸増幅検査 (nucleic acid amplification testing:NAT) で陽性となった例のうち，HBc 抗体が陰性で感染初期と考えられる 739 例では，遺伝子型 C 502 例 (67.9%)，遺伝子型 A 144 例 (19.5%)，遺伝子型 B 81 例 (11.0%)，遺伝子型 D 7 例 (0.9%)，遺伝子型 H 4 例 (0.5%)，遺伝子型 E 1 例 (0.1%) であった. 感染初期例では遺伝子型 A の検出頻度が著しく高くなっており，逆に遺伝子型 B はキャリア報告例と同程度の頻度となっていた. 感染初期例の遺伝子型 A を更に詳しく調べると大多数は欧米型の遺伝子型 Ae であり，外国型 HBV が我が国で蔓延している様子が窺える. キャリア例の多くは 16 年以上前に母子感染や乳幼児期の注射器の使い回しなどによって感染したと考えられ，現在の感染初期例のほとんどは性感染と考えられている. HBV は免疫機能が未発達な乳幼児期に感染するとキャリア化する確率が高く，成人に達してからの感染では前述のように一過性の経過で治癒すると考えられてきた. しかし遺伝子型 A 感染は成人でも約 10% がキャリア化するといわれ [4]，キャリア化した人からの二次感染により感染が拡大していると考えられる.

◆ 4. 我が国における輸血後肝炎の推移

輸血用血液が売血によりまかなわれていた 1960 年代には，輸血を受けた患者の約半数が肝炎を起こしていたと報告されている. 1969 年に献血制度が確立され輸血後肝炎発症率は 16.2% に低下した. その後 HBs 抗原検査の導入，400ml 献血・成分献

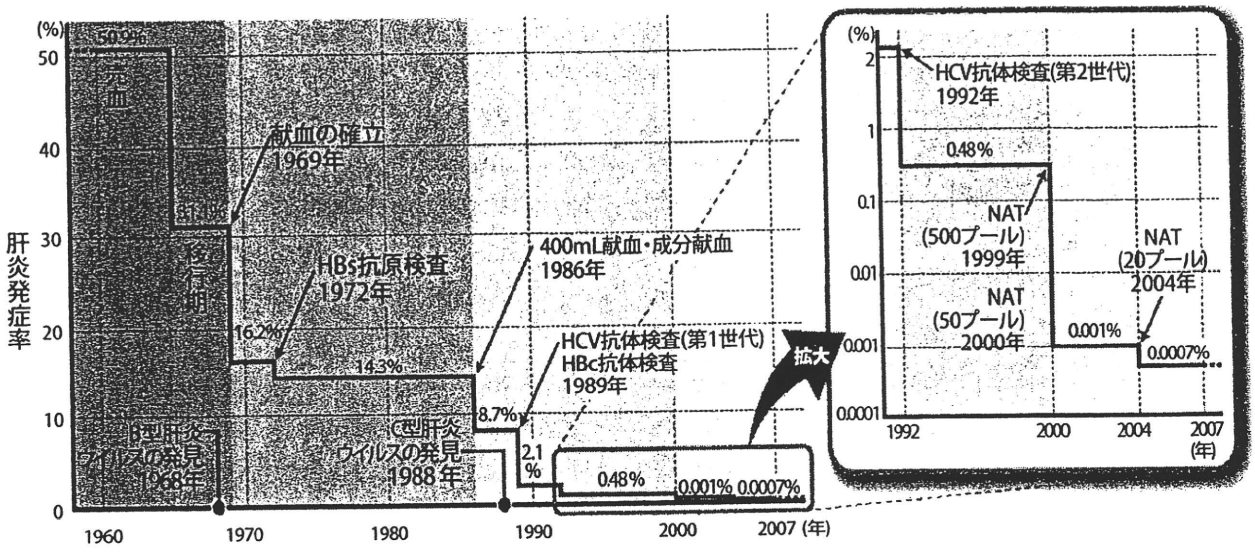

図3　我が国における輸血後肝炎の推移（『輸血情報 0811-116』）

血の導入やHCV抗体検査・HBc抗体検査などの新たな検査の導入により，1992年には0.48%にまで低下した．1999年にはNATがすべての輸血用血液に対して導入され，発症率は0.001%にまで低下した．その後もプールサイズを当初の500プールから現在の20プールにまで縮小，検査機器・試薬の改良もあって発症率は現在0.0007%と推定されている（**図3**）．ただし，かつては「輸血後2週以降6ヶ月の間に，S-ALT(S-GPT)が100IU/L以上の肝機能異常が初発し，継続的に2週以上に及んだ場合，輸血後肝炎と診断する」との診断基準で判断されていたため，原疾患に起因するALTの上昇や術後肝障害・薬剤性肝障害・肝炎ウイルス以外のウイルス感染などによる肝障害が含まれていたと考えられる．

表1　輸血後肝炎の推移

		HBV	HCV
before	1997	12	1
NAT	1998	22	7
	1999	21	5
	2000	5	0
	2001	7	0
	2002	8	0
after	2003	12	0
NAT	2004	20	0
	2005	11	1
	2006	6	1
	2007	13	1
	2008	4	0

◆5. 輸血後肝炎の現状

新しい検査法の導入など，さまざまな安全対策の実施により輸血用血液の安全性は非常に高くなっている．特に，HBV，HCVおよびHIV-1を対象ウイルスとして1999年に導入されたNAT開始以降HBV，HCVの輸血感染は大きく減少した（**表1**）．NAT導入前ではHBVで20例以上/年，HCVで5～7例/年の輸血感染が確認されていたが，導入後の2000年から2002年ではHBVで5～8例/年に減少し，HCVの輸血感染は確認されなかった．2004年8月から徹底した遡及調査が開始され，過去にまで遡って調査を行ったため2003年，2004年はHBVの感染例が増加している．遡及調査とは，「病原体の存在が疑われた献血者の過去の献血血液または輸血等により感染が疑われた血液製剤等に関する情報及びこれらの献血血液から製造された血液製剤の情報，当該製剤が投与された患者の感染に係る情報等を収集し，それを科学的に分析・評価することである」と定義されている．それまでは輸血後の患者さんに肝炎等の発症が疑われ，その情報が医療機

関から報告された場合に輸血感染の調査が行われていたが，遡及調査開始後は①過去の献血で検査結果が陰性であった献血者が陽転した場合は過去の献血血液にまで遡って調査，②輸血後の患者さんに肝炎等の発症が疑われた場合は，使用されたすべての輸血用血液を対象として調査を行っている．2005年から2007年まで1例ずつHCV感染が確認されているが，この3例中2例は遡及調査によって判明した事例である．これらの調査が可能となったのも，1996年9月から献血時の血液の一部を「保管検体」として凍結保存しているためで，現在の規定では11年間の保存期間となっている．2008年にはHBV4例の輸血感染が確認されているが，その中の1例は当該血液が個別NATでもHBV-DNAが検出されない感染極初期の血液が原因となっていた．この事例では当該献血者の次回献血血液中のウイルスと患者さんの発症時のウイルスの相同性調査により確定された．このような血液による感染は現在の最新技術を駆使しても防ぐことは困難である．感染機会のあった献血者をどのようにして排除するかが問題となっている．

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Original Article

The associated markers and their limitations for the primary screening of HCV carriers in public health examination

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Aim: Although the anti-hepatitis C virus (HCV) antibody test has been recommended to the whole Japanese population, most countries have not implemented it. The present study aims to re-evaluate the usefulness of markers examined in the general health examination for the initial screening of HCV carriers.

Methods: Of the overall population, 25 142 individuals (8876 males, 16 266 females) participated in health examinations with HCV tests in 2005, and the most commonly associated markers for HCV-positive subjects were explored by multivariate analysis, based on blood biochemical, physical, sphygmomanometric and hematological parameters. Thereafter, the efficiencies of the markers were estimated from a total population of 85 013 individuals (29 502 males, 55 511 females) in 2003–2005.

Results: The most significantly associated markers for HCV positivity were aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Optimal limits of ALT and AST by receiver–operator characteristic (ROC) analysis were 24 and 27 IU (male, 33 and 28 IU; female, 22 and 26 IU), respectively. However, one-quarter of HCV carriers were not found to be positive using the optimal limits of aminotransferases.

Conclusion: The present study confirmed the limitation of serum aminotransferase levels as markers of HCV for primary screening. Therefore, at present, an anti-HCV antibody test is required for the efficient screening of HCV carriers in all health examinations.

Key words: aminotransferases, HCV, health examination

INTRODUCTION

INFECTION WITH HEPATITIS C virus (HCV) has been the leading cause of liver cirrhosis, and the consequent development of hepatocellular carcinoma, for the past few decades. The number of HCV carriers has increased worldwide. Indeed, the World Health Organization (WHO) estimates that about 180 million people, that is 3% of the world's population, are infected with HCV, and 3–4 million people are newly infected every year, 70% of whom develop chronic hepatitis.[1]

Based on early detection and treatment, it is very important to detect HCV carriers as early as possible, for example, in public health examinations. HCV carriers are diagnosed by the detection of HCV-RNA and/or anti-HCV antibody using the judgment system of HCV infection established since 2002 in Japan (Fig. 1). Generally, subjects who have abnormally high levels of serum alanine aminotransferase (ALT) as well as aspartate aminotransferase (AST) are recommend to take thorough examinations for liver diseases, including the HCV tests. However, there is an issue that most HCV carriers are considered to be asymptomatic and paucisymptomatic, and that approximately 30% of chronic HCV carriers persistently exhibit normal ALT levels (PNAL), while another 40% exhibit minimally elevated ALT levels.[2–6] Consequently, these asymptomatic and paucisymptomatic HCV carriers fail to be detected by the primary screening using serum ALT levels in public health examinations. Importantly, these asymptomatic HCV carriers with PNAL have significant histological

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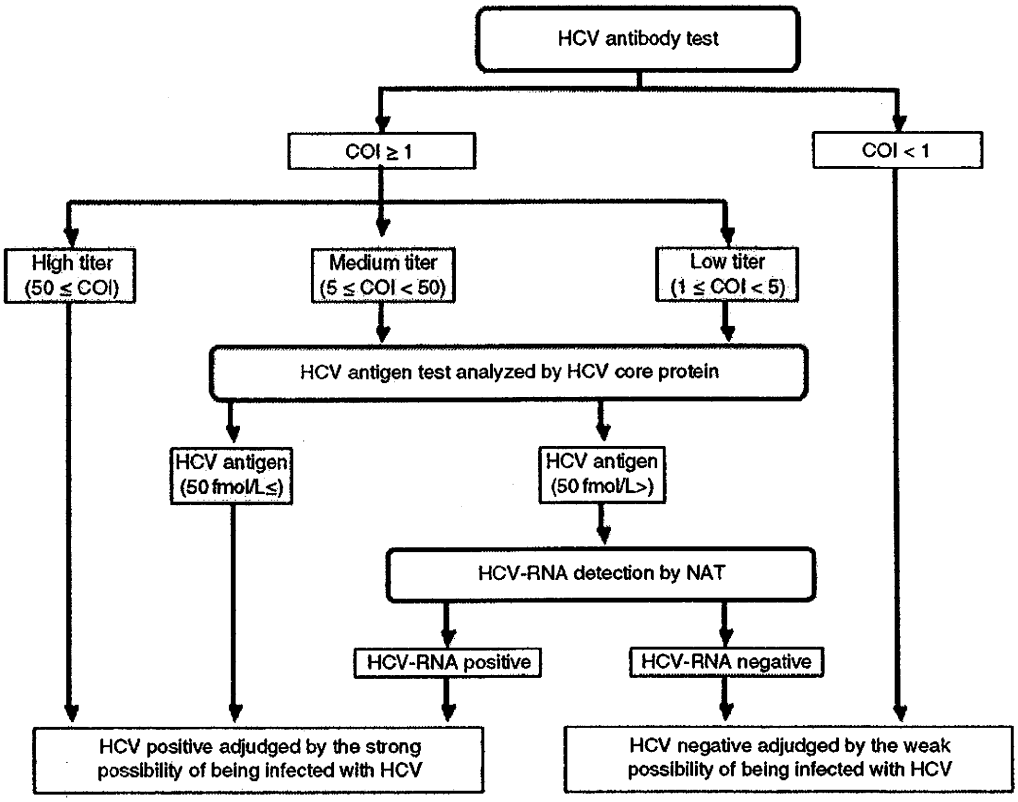

Figure 1 Flow chart showing the course of medical examination for hepatitis C virus (HCV). The diagnosis of HCV infection was conducted in accordance with the guidelines for the medical examination of HCV issued by the Japanese Ministry of Health, Labour and Welfare. COI, cut off index; HCV, hepatitis C virus; NAT, nucleic acid amplification test.

liver damage, similar to that in HCV carriers with raised ALT levels, and moderate to severe hepatitis has frequently been found in asymptomatic HCV carriers compared with HCV carriers with raised ALT levels.[7]

Accordingly, the optimal serum ALT limits for the screening of HCV carriers have been subject to debate.[8–10] However, what is considered a healthy range of ALT levels compared with liver disease differs between medical institutes, centers, hospitals, regions and countries. Almost all of the normal ALT ranges for liver disease are less than 40 IU,[7] however, Prati *et al.* reported that the upper limits of the healthy range differed between genders; 30 IU and 19 IU for males and females, respectively; calculated as the value of the 95th percentile in normal subjects from a population at the lowest risk for liver disease.[8] Furthermore, Okanoue *et al.* defined asymptomatic HCV carriers as those with PNAL less than 30 IU based on the histological fibrosis stage in a follow-up study.[11] In Japan, serum ALT levels under 35 IU had been considered to be within the healthy limit for diagnosis of liver diseases, but in 2008, the health limit was reduced to under 30 IU, for both ALT and AST, as suggesting liver disease in public health examinations, based on the guidance for antivirus therapy of HCV.[12] Based on these facts, it has not been actually clarified whether these markers are effective and whether the optimal limit points are useful or not for the detection of asymptomatic and paucisymptomatic HCV carriers. Therefore, in Japan, the anti-HCV antibody test has been recommended to the whole of the population during public health examinations.[13,14]

The purpose of the present study was to re-evaluate the effectiveness of serum aminotransferase levels as markers in the primary screening for HCV carrier detection in over 85 000 subjects in the annual public medical health examination for 3 years between 2003 and 2005.

METHODS

Population in the health examination

A TOTAL OF 85 013 individuals (29 502 males, 55 511 females), including non-employees, local residents, self-employed persons, farmers, housewives and retired persons participated in the biochemical examination of serum ALT and AST levels as part of the annual public health examination and HCV testing during the 3 years from 2003 to 2005 in Ibaraki Prefecture, Japan. HCV testing was carried out based in part on a project of urgent comprehensive countermeasures against hepatitis and HCC at the ages of 40, 45, 50, 55, 60, 65, or 70 for five years supported by the Japanese Ministry of Health, Labour and Welfare. In the health examination in 2005, in addition to the measurement of serum ALT and AST levels, 25 142 subjects (8876 males, 16 266 females) underwent examination of γ-GFP level, diastolic and systolic blood pressure, hemoglobin, hematocrit, red blood cell count (RBC), total cholesterol, triglyceride, glucose and glycohemoglobin (HbA_{1c}). Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters.[15] All of the health examinations with serum biochemical analyses, as well as the HCV tests, were carried out with the Ibaraki Health Service Association (Mito, Japan).

The determination of HCV carrier status

The determination of the presence or absence of HCV infection was performed in accordance with the guidelines for the medical examination of HCV issued by the Japanese Ministry of Health, Labour and Welfare, as summarized in Figure 1. Serum collected from the subjects during the medical examination was first measured for the HCV titer using a chemiluminescent enzyme immunoassay for HCV antibody (Lumipulse®, Fujirebio Inc, Tokyo, Japan). Subjects with serum HCV titer beneath a cut-off index (COI) of 1 were determined to be HCV negative. Those subjects with a COI > 1 were divided into three classes dependent on the levels of the HCV titer: low titer, COI under 5 and more than 1; medium titer, COI under 50 and more than 5; high titer, COI more than 50. The subjects in the high titer class were determined to be HCV positive. The subjects classified to the low and medium titers underwent the HCV antigen test analysis for the HCV core protein. Subjects with more than 50 fmol/L of HCV antigen were determined to be HCV positive. When the HCV antigen was under 50 fmol/L, a nucleic acid amplification test (NAT) was conducted for HCV-RNA detection. The subjects with positive and negative results by the NAT were finally determined to be HCV positive and negative, respectively.

Other investigated data in 2005

In the data from 2005, the most relevant factor for HCV positive status was determined statistically by multivariate analysis and the ROC curve. As a result of the ROC curve in 2005 (Table 1), the ROC curves for ALT and AST levels in serum were drawn from data for 3 years between 2003 and 2005 to evaluate the effective cut-off points to avoid false negative and positive findings for HCV.

Statistical analysis

Data are presented as the mean ± SE, the percentage and the percentiles. Significant differences were determined by unpaired Student's *t*-test or one-way ANOVA with Bonferonni's post-hoc test for comparisons between two groups or among multiple groups, respectively. The statistical analysis was performed using SPSS II software version 11.0 (SPSS Inc, Chicago, IL, USA). Multiple regression analyses were made using the stepwise method. The upper-left cut points for the HCV positive were chosen from likelihood value based on the ROC curve. ROC comparison was performed by calculation of the area under the curve and 95% confidence intervals using the technique described by Hanley and McNeil.[16]

RESULTS

Basic data, and the ROC and multivariate analyses of the health examinations in 2005

BASIC DATA OF all examined parameters in the health examinations in 2005 are shown in Table 2. The levels of serum ALT, AST and γ-GPT were significantly and markedly higher in the HCV positive than in the HCV negative subjects, for both genders. These serum levels were significantly higher in males than in females in all of the HCV negative and positive cases.

Table 1 presents the results of ROC and multivariate analyses in the respective parameters for HCV positive status from data in 2005. The most significant relevant parameter for HCV positive status was the serum AST level, followed by the serum ALT level. There were other significant parameters ($P < 0.05$), but the areas of the ROC curve for these parameters were less than 0.7. BMI and serum levels of triglyceride and total cholesterol

Table 1 Area under the receiver-operator characteristic (ROC) curve and multivariate analysis and the respective parameter for HCV positive subjects examined in 2005

Parameter	ROC curve area	SE	*P*-value	95% CI
AST	0.849	0.018	0.000	0.814–0.884
ALT	0.788	0.021	0.000	0.747–0.829
Hemoglobin	0.654	0.028	0.000	0.598–0.709
Age	0.642	0.026	0.000	0.591–0.692
Hematocrit	0.642	0.027	0.000	0.589–0.695
γ-GTP	0.622	0.028	0.000	0.508–0.677
Glucose	0.613	0.025	0.000	0.564–0.662
RBC	0.558	0.029	0.029	0.501–0.614
Systolic pressure	0.547	0.029	0.077	0.491–0.603
Diastolic pressure	0.528	0.028	0.289	0.473–0.583
Height	0.519	0.027	0.474	0.466–0.572
Weight	0.505	0.027	0.860	0.451–0.558
HbA1c	0.504	0.028	0.872	0.449–0.559
BMI	0.492	0.025	0.760	0.443–0.457
Triglyceride	0.409	0.025	0.001	0.361–0.330
Total cholesterol	0.278	0.027	0.000	0.226–0.330

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CI, confidence interval; HbA1c, glycohemoglobin; RBC, red blood cell count; γ-GTP, gamma-glutamyl transferase.

were related to HCV negative status but not HCV positive status. In particular, the total cholesterol level was the most relevant parameter for the HCV negative status. Based on the results of these analyses, the ROC curves for the serum AST and ALT levels of the HCV positive subjects among each gender were drawn from data for 3 years between 2003 and 2005.

As result of stepwise discrimination analysis, a combination of four parameters, AST, ALT, age and total cholesterol, gave the maximum likelihood. The established discrimination formula was as follows: $Z = 10.472 - 0.001 \times (\text{AST, IU}) + 0.027 \times (\text{ALT, IU}) + 0.057 \times (\text{age, year}) - 0.025 \times (\text{total cholesterol, mg/dL})$. However, the calculated predictive value for HCV positive ratio was only 6.61% using the formula.

HCV positive ratio and distribution of aminotransferases in HCV positive populations for 3 years

There were 787 HCV positive subjects (male, 406; female, 381) and the positive ratio was 0.93% (male, 1.38%; female, 0.69%) for 3 years between 2003 and 2005. The range of ages for the HCV positive subjects was 29–87 years old (male, 29–87 years; female, 40–84 years).

The distributions of serum ALT and AST levels were expressed as percentiles by age in the HCV positive populations for both genders (Fig. 2). In males, the levels of both aminotransferases, in particular ALT, were elevated in those aged less than 65 years, and there were large variations of these levels in all age ranges (Fig. 2a). However, there were no differences in the distribution of levels of both aminotransferases among the age ranges in females, and the variations of these levels were small compared to those in males (Fig. 2b).

The distribution of HCV positive subjects using the current limit points

Those who were detected as being HCV positive in 2003–2005 were divided into four cut-off ranges (A–D) by ALT and AST levels at 30 IU, that is the current limit point (Fig. 3). There was significant difference in the ratio balance of HCV positive between genders assessed by χ^2 analysis ($P < 0.0001$). In range A, which means the false-negative of HCV positive, the HCV positive rates of male and female were 25.9% and 47.0%, respectively. In range A, almost of half of female HCV positive subjects were classified as false-negative. In contrast, the ratios in range D were 56.3 and 39.8% in males and females, respectively, and over half of HCV positive subjects in males were included in range D. In males, the ratio in range B was 10.5% and higher than that (7.4%) in range C. In contrast, in females, the ratio in range B was only 4.3% and lower than that (9.0%) in range C.

Table 2 Basic data in all examined parameters in 2005

	Male				Female						
	Total	HCV (−)	HCV (+)	†	Total	‡	HCV (−)	‡	HCV (+)	†	‡
Population number	8876	8776	100		16266		16178		88		
ALT (IU/L)	27.2 ± 0.2	26.8 ± 0.2	68.0 ± 7.0	***	19.7 ± 0.1	***	19.6 ± 0.1	***	45.3 ± 0.1	***	**
AST (IU/L)	26.4 ± 0.1	26.0 ± 0.1	57.1 ± 4.3	***	22.5 ± 0.1	***	22.4 ± 0.1	***	46.9 ± 0.1	***	**
γ-GTP (IU/L)	53.6 ± 0.7	53.1 ± 0.7	101.1 ± 14.3	***	25.9 ± 0.2	***	25.9 ± 0.2	***	39.8 ± 0.2	*	***
Height (cm)	165.0 ± 0.1	165.0 ± 0.1	164.3 ± 0.6	ns	153.3 ± 0.0	***	153.3 ± 0.0	***	151.7 ± 0.0	*	***
Weight (kg)	65.3 ± 0.1	65.3 ± 0.1	63.7 ± 1.0	ns	54.3 ± 0.1	***	54.3 ± 0.1	***	53.4 ± 0.1	ns	***
BMI	24.0 ± 0.0	24.0 ± 0.0	23.6 ± 0.3	ns	23.1 ± 0.0	***	23.1 ± 0.0	***	23.2 ± 0.0	ns	ns
Systolic pressure (mmHg)	128.5 ± 0.2	128.6 ± 0.2	126.9 ± 2.0	ns	124.1 ± 0.1	***	124.0 ± 0.1	***	128.6 ± 0.1	*	ns
Diastolic pressure (mmHg)	81.1 ± 0.1	81.1 ± 0.1	78.7 ± 1.3	*	74.5 ± 0.1	***	74.5 ± 0.1	***	76.7 ± 0.1	ns	ns
Hemoglobin (g/dL)	32.0 ± 0.0	32.0 ± 0.0	32.3 ± 0.2	*	30.6 ± 0.0	***	30.6 ± 0.0	***	31.6 ± 0.0	***	*
Hematocrit (%)	95.5 ± 0.1	95.5 ± 0.1	96.2 ± 0.6	ns	91.7 ± 0.0	***	91.7 ± 0.0	***	94.3 ± 0.0	***	*
RBC ($\times 10^4$ cells/μL)	33.5 ± 0.0	33.5 ± 0.0	33.5 ± 0.1	ns	33.4 ± 0.0	***	33.4 ± 0.0	***	33.5 ± 0.0	ns	ns
Total cholesterol (mg/dL)	200.0 ± 0.4	200.4 ± 0.3	170.6 ± 3.1	***	210.6 ± 0.3	***	210.7 ± 0.3	***	191.1 ± 0.3	***	**
Triglyceride (mg/dL)	143.8 ± 1.2	144.0 ± 1.3	119.7 ± 12.3	***	110.5 ± 0.5	***	110.6 ± 0.5	***	101.2 ± 0.5	ns	ns
Glucose (mg/dL)	107.3 ± 0.3	107.2 ± 0.3	118.2 ± 4.2	*	99.3 ± 0.2	***	99.3 ± 0.2	***	108.9 ± 0.2	*	*
HbA_{1c} (%)	5.2 ± 0.0	5.2 ± 0.0	5.3 ± 0.1	ns	5.1 ± 0.0	***	5.1 ± 0.0	***	5.1 ± 0.0	ns	ns

Data are shown as mean ± SE. Significant differences were analyzed by the non-parametric Mann–Whitney *U*-test; $^*P < 0.05$, $^{**}P < 0.001$, $^{***}P < 0.0001$. †: HCV (−) versus HCV (+), ‡: male versus female.

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; HbA1c, glycohemoglobin; ns, no significance; RBC, red blood cell count; γ-GTP, gamma-glutamyl transferase.

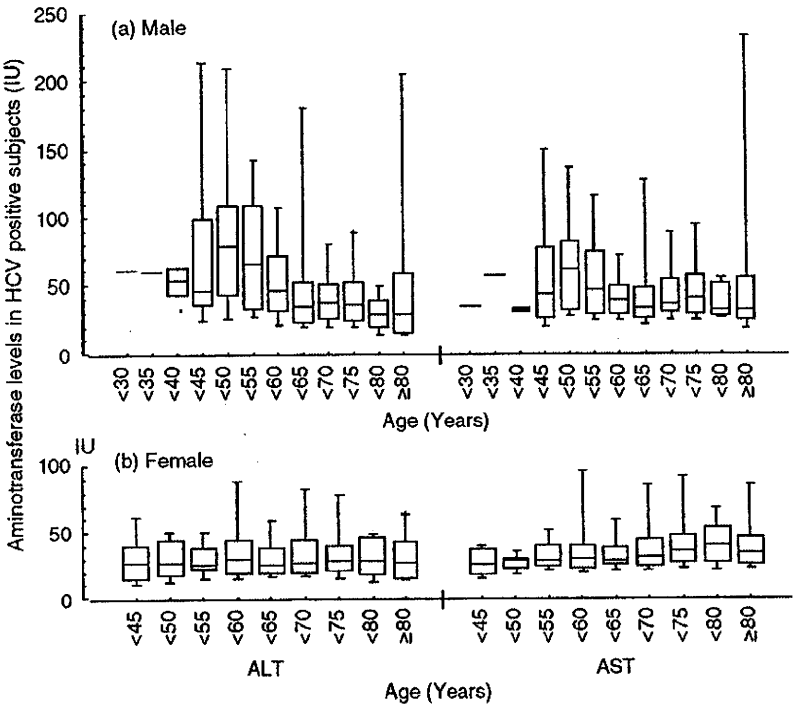

Figure 2 Distributions of serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the respective age ranges of hepatitis C virus (HCV) positive populations. The age ranges were divided into 5-year increments in males (a) and females (b). Data are shown as the 10th, 25th, 50th, 75th and 90th percentiles by box and whisker plots.

Optimal limits of aminotransferases by ROC curve

The ROC curves of serum ALT and AST levels for HCV positive subjects are shown by gender for 3 years in Figure 4. For both genders, the ROC curves for AST were positioned to the upper-left compared with those for ALT (Fig. 4a). The cut-off point (threshold) values in the uppermost left position on the curve were 24 IU (sensitivity 0.727: 1-specificity 0.280) and 27 IU (0.717: 0.222) for ALT and AST, respectively. Even in cases divided by gender, the ROC curves for AST for the respective gender was to the upper-left compared with those for ALT. In particular, the lower curve for ALT was remarkable compared to that for AST in males. The cut-off point values in the uppermost left (threshold) in males were 33 IU (sensitivity 0.577: 1-specificity 0.210) and 28 IU (0.761: 0.284) for ALT and AST, respectively, and in females were 22 IU (0.697: 0.256) and 26 IU (0.692: 0.202) for ALT and AST, respectively. These threshold values were defined as the proposed limit points in the present study.

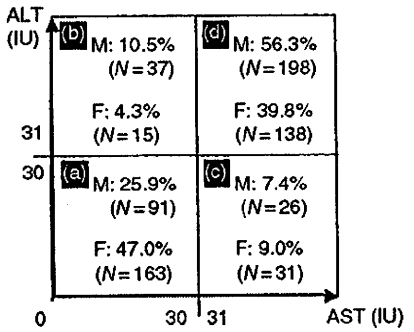

Figure 3 Hepatitis C virus (HCV) positive ratios by gender in the ranges cut off by the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) at 30 IU. Data are shown as the percentage of HCV positive for the total HCV positive in each gender. The ranges less than 30 IU of both ALT and AST show the false-negative of HCV positive. χ^2 value = 40.836, $P < 0.0001$. The numbers in parenthesis show the HCV positive subjects. F, female; M, male; *N*, number.

Comparison of efficiency (sensitivity and specificity) among the three different limit points

The efficiency of the proposed limit points for serum ALT and AST levels were compared to those for the previous and current limit points in Japan based on the ratios of the true-positive and false-negative in the HCV positive

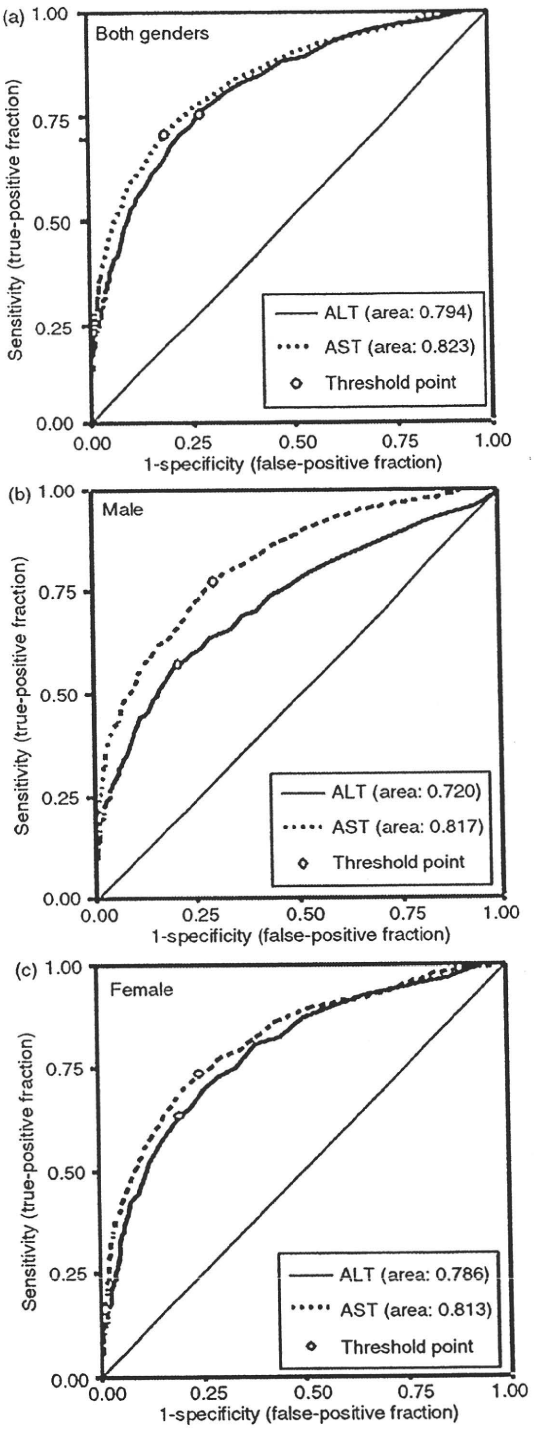

Figure 4 Receiver–operator characteristic (ROC) curves of sensitivity (true-positive fraction) plotted against 1-specificity (false-positive fraction) for serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the subjects that were diagnosed as hepatitis C virus (HCV) positive in examinations over 3 years for both genders (a) and by gender (male in (b), female in (c)). The cut-off point (threshold) values in the uppermost left of the curve (sensitivity: 1-specificity): both genders, ALT 24 IU (0.727: 0.280), AST 27 IU (0.717: 0.222); male, ALT 33 IU (0.577: 0.210), AST 28 IU (0.761: 0.284); female, ALT 22 IU (0.697: 0.256), AST 26 IU (0.692: 0.202). Area, area under the ROC curve.

group and of the true-negative and false-positive in the HCV negative group: the previous (ALT ≤ 35/AST ≤ 40), the current (ALT ≤ 30/AST ≤ 30) and the proposed limit points, by both genders (Fig. 5a) and each gender (males in Fig. 5b, females in Fig. 5c). For example, in case of the HCV positive group, the true-positive and false-negative ratios show the proportions of the HCV positive group outside and within the ranges, respectively, divided by the respective limit point.

As shown in Fig. 5, the true-positive and true-negative ratios were increased and decreased, respectively, depending on the limit points with the lower aminotransferase values. In both genders, shown in Fig. 5a, the true-positive ratio was improved to 76.7% with the proposed limit points. However, one-quarter of the HCV positive cases were judged as false-negative. In contrast, in the case of the HCV negative ratio, the true-negative ratio was decreased from 89.8% with the previous limit point to as low as 70.0% with the proposed limit point. Although the sum of true-positive and true-negative ratios with the proposed limit point was the highest among these cut-off points, the sum ratio was only 146.7%.

Likewise, in the case of males, shown in Fig. 5b, the true-positive ratio was improved with the proposed limit point, but only three-quarters. However, the true-negative ratios were decreased from 82.4% with the previous limit points to 69.9% with the proposed points. There were no marked differences in these ratios between the current and proposed limit points. In females, shown in Fig. 5c, the true-positive ratio was as low as 40% with the previous limit point. Although the true-positive ratio was remarkably improved with the proposed limit point, the ratio was only about 70%. In contrast, the true-negative ratio was as high as 94% with the previous limit point, but was down to three-quarters with the proposed limit point.

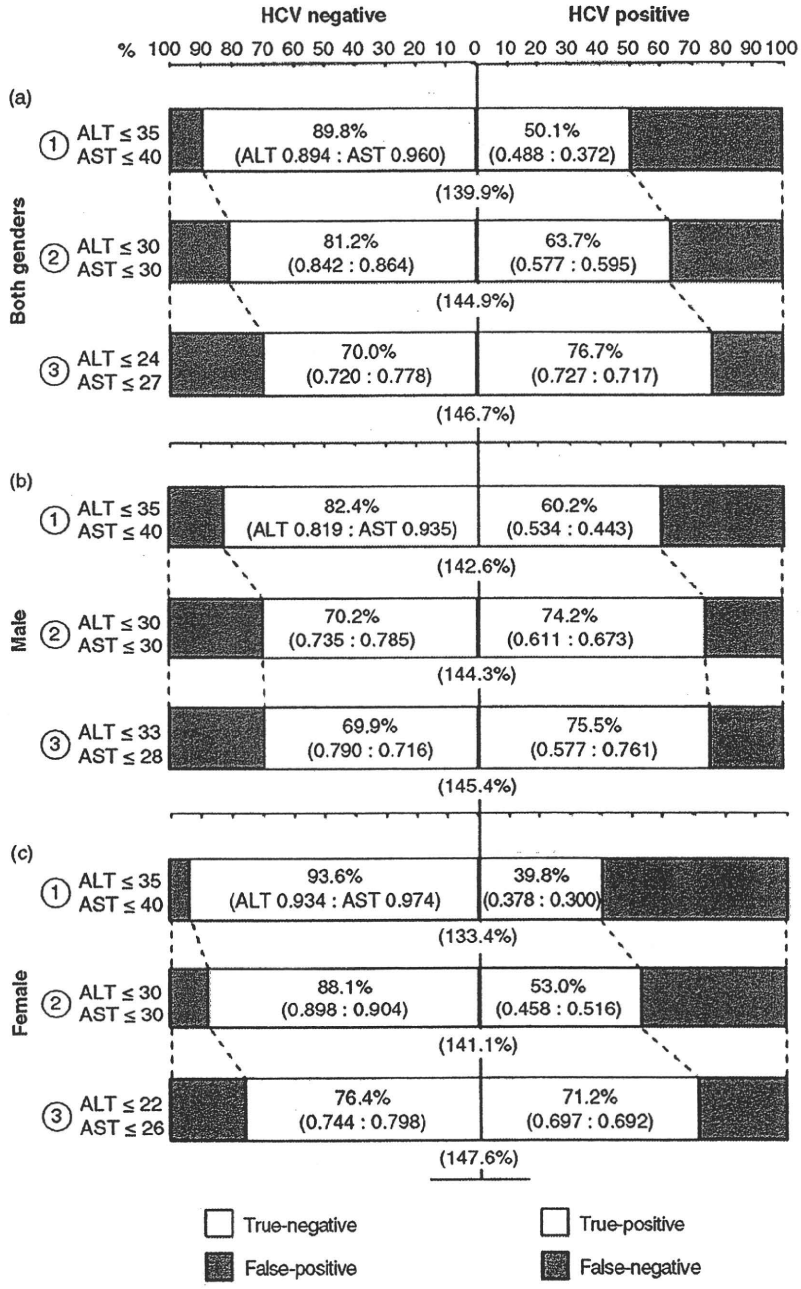

Figure 5 Percentages of true-positive among the HCV positive and true-negative among the HCV negative divided by the three set limit points of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. (1) The previous limit points; (2) the current limit points; and (3) the proposed limit points. The true-negative and false-negative: the negative and positive ratios for HCV within the range set by the respective limit points. The false-positive and true-positive: the negative and positive ratios for HCV outside the range set by the respective limit points. The values in parentheses in the positive and negative columns indicate sensitivity (true-positive) and specificity (true-negative), respectively. In addition, the values in parentheses beneath the columns indicate the sum of the true-positive and true-negative.

DISCUSSION

THIS STUDY AIMED to re-evaluate the effectiveness of serum aminotransferases levels as a marker in the primary screening for HCV carrier detection in over 85 000 subjects in the annual public medical health examination. Before the evaluation, the optimal parameter obtained during public health examinations was also elucidated for HCV positive. Consequently, serum aminotransferases levels were confirmed as the optimal parameters related to an HCV positive diagnosis by the judgment system for HCV infection. Serum AST levels were more significantly associated with HCV positivity than ALT in both males and females. Furthermore, the optimal limit points (proposed limit points) for the healthy range derived from the ROC curve were different between genders (male, ALT33/AST28; female, ALT22/AST26). Based on a comparison of the proposed limit points to the previous (ALT ≤ 35/AST ≤ 40) and the current (ALT ≤ 30/AST ≤ 30) limit points in Japan, the efficiency of the proposed limit points was superior to the others. However, in males, 23.3% of HCV carriers were considered as negative (false-negative), although the true-positive ratio was increased by 15.3% from the previous limit point to the proposed limit point. In females, the true-positive ratio for the proposed limit point was markedly elevated by 31.4% compared to the previous limit point. Importantly, at least one-quarter of HCV carriers could not be captured for each of the genders, even when using these optimal conditions. Likewise, the incidence of false-positive among the HCV negative cases increased to 30 and 25% in males and females, respectively, with the optimal limit points. Therefore, one limitation of the use of aminotransferase levels as an indicator for HCV screening was found due to these low efficiencies. These results mean that almost one-quarter of male and half of female HCV carriers are already dropped from the thorough examinations and therapies when using the current limit points of serum aminotransferase levels.

Conry-Cantilena *et al.* have reported that 31 and 42% of HCV carriers exhibited PNAL (under ALT 41 IU) and minimally raised ALT levels (peak level of ALT less than 80 IU), respectively, in a follow-up study for 5 years.[2] The present study also demonstrated that the proportion of HCV carriers with PNAL (less than ALT 30 IU) was 33.3 and 56.0%, in males and females, respectively (Fig. 3). Although it is very important to determine how so many HCV carriers with PNAL can be screened in the health examinations, one of the markers associated with HCV positive status in the present study was ALT. Therefore, there are limitations to the use of ALT levels to screen for HCV with PNAL.

As the present study showed that AST rather than ALT levels were associated with HCV positive status, a significant relationship has been reported between AST, but not ALT, and liver damage, including portal inflammatory piecemeal necrosis and liver fibrosis.[17,18] In addition, Schiffman *et al.* observed that there was no correlation between baseline ALT activity, HCV-RNA level and liver histology in HCV patients with PNAL.[6] Puoti *et al.* described ALT levels as possibly being less important for the determination to undergo therapy than other factors, such as age, HCV genotype, liver histology, patient motivation, symptoms, extra-hepatic manifestations and co-morbid illnesses.[19] For hepatic fibrosis patients with not only HCV but also several other liver diseases, serum AST level has been suggested to be a better marker than ALT.[20–22] Assy and Minuk suggested possible reasons to explain why AST, but not ALT values, correlate with histological findings in patients with HCV.[18] One of the possible reasons is that co-existing non-viral-related fatty infiltration of the liver might contribute disproportionately to the elevation of ALT. Another is that HCV might destroy mitochondria, where AST exists as a specific enzyme, resulting in intracellular trophism. In addition, AST activity may be more stable than ALT activity.[23] However, it remains unclear why AST values correlate with hepatic histological features, in particular fibrosis, and further studies are therefore needed. Indeed, while there is no doubt that both ALT and AST are useful parameters for liver disease,[24] there are limitations of the use of ALT and AST as parameters for HCV screening in public health examinations.

In the present study, the serum aminotransferase levels were different between the genders, in agreement with previous studies.[7–9] Prati *et al.* indicated different normal ranges of ALT for liver disease between males and females (30 and 19 IU, respectively) based upon calculation of the 95th percentile of serum ALT levels in normal subjects with a population at lower risk for liver disease.[8] In general, both serum aminotransferase levels in females tend to be lower than those in males.[7,25–30] Puoti *et al.* speculated that different hormonal factors, such as estrogens, could act as modulators of some mechanisms and may explain the differences between genders,[25] based on the decreased ALT levels in pregnant and estrogen-treated females with chronic hepatitis C.[31,32] Indeed, in the present study, the aminotransferase levels in HCV positive females were independent of age; although these levels, especially ALT, were elevated in males aged less than 65 years. The current results may be

supported by these reports. Therefore, the cut-off points of ALT and AST might need to be lower than ALT in females, particularly in the younger age groups, for HCV screening.

The present study attempted to generate a useful equivalent for the detection of HCV carriers by logistic analysis. Ultimately, the equivalent, based on some significant parameters associated with HCV carriers led from the stepwise analysis shown in Table 2, could not efficiently detect HCV positive rates below 10%. Therefore, in the present study, the equivalent was not an effective tool for the screening of HCV carriers. In future, a more effective logistic equivalent should be considered using better parameters, including platelet counts,[9,12] in a larger population of both public and clinical examinations for the screening of HCV carriers, because the present judgment system for HCV infection needs some steps added with different specific equipment and techniques and the consequent costs. The effective logistic equivalent from the parameters used in public examination will be a good way from the viewpoint of cost effectiveness and will be available in developing countries. However, at present, there are no highly efficient methods that can be applied to the health examination for the screening of HCV carriers. Furthermore, as shown by the results of the present study, one-quarter of HCV carriers, in particular almost half of female carriers, have been diagnosed as healthy subjects and, therefore, have missed out on any treatments for HCV infection. This fact supports the recommendation of HCV tests, at least the anti-HCV antibody test, to all individuals during health examinations for the early detection of asymptomatic HCV carriers; this should be more important than cost considerations.

In conclusion, the limitation of serum aminotransferase levels as markers of HCV carriers for primary screening in public medical examinations was confirmed, because one-quarter of the HCV carriers were categorized as healthy subjects. Therefore, the current investigation supports the necessity to apply HCV testing to all individuals undergoing public medical examinations for early detection and early treatment and to prevent the spread of HCV.

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