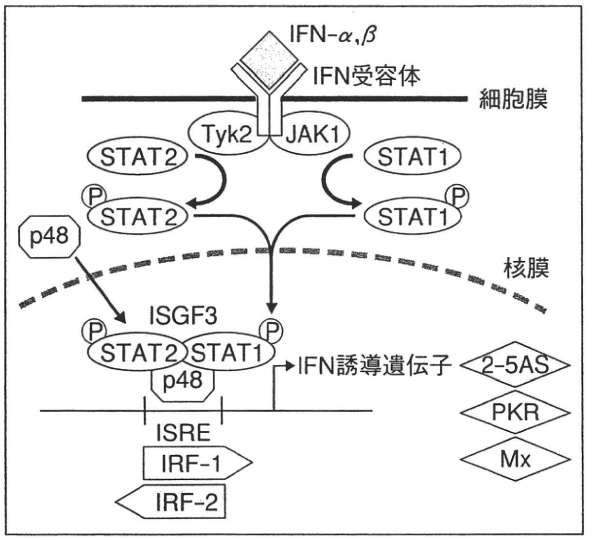

図 1　インターフェロン（IFN）シグナルの伝達経路

ることが可能である。

抗ウイルス蛋白である 2-5AS や PKR の発現は上流に位置する ISRE によって制御されており，このような転写調節因子の発現には個体差が存在することが予想される。また，IFN シグナル伝達経路である JAK-STAT 経路のネガティブ・フィードバック機構として STAT の活性を抑制する cytokine-inducible SH2 protein (CIS) や JAK に結合してそのシグナルの発現を遮断する JAK-binding protein の存在も報告され，これらの IFN 発現遺伝子を制御する因子が IFN 治療効果に関連する可能性がある。

報告されている IFN 関連遺伝子と IFN 治療効果についてはまず，IFN 受容体に関するものがあげられる。IFN が結合する受容体としては IFNAR1 と IFNAR2 があり，前者は IFNα のサブタイプのひとつである IFNα8 にのみ結合し，後者はすべての IFNα と IFNβ に結合することが知られている。この IFN 受容体 mRNA の肝での発現量が高い症例は IFN 療法の効果が高いことが報告されており[2,3]，これは肝での IFNAR2 の蛋白レベルでの発現においても確認されている[4]。また逆に，可溶性 IFNAR2 の発現量が多い症例は IFN の効果が低く[5]，これは IFNα がレセプターに結合するのと拮抗するためと考えられている[6]。また，肝細胞 IFN シグナル関連 mRNA では，JAK-binding protein の発現が高い症例で IFN 著効例が多いと報告されている[7]。

表 1　末梢血リンパ球中の遺伝子発現の組み合わせによるインターフェロン（IFN）治療効果の予測（文献 19 より引用改変）

IFN 投与前 末梢血リンパ球中の遺伝子の組み合わせ	精度，% training	test
1 topoisomerase（DNA）I	53.9	46.2
2 catenin（cadherin-associated protein）β1（88 kDa）	66.0	57.1
3 Ras-related C3 botulinum toxin substrate 2	91.0	89.1
IFN 投与開始 2 週間後 末梢血リンパ球中の遺伝子の組み合わせ	**精度，% training**	**test**
1 differentiation 6（septin 2）	28.8	25.8
2 cyclin G1	75.0	64.4
3 cell division cycle 20 homolog（*Saccharomyces cerevisiae*）	90.2	87.9
1 MIHC	28.8	25.0
2 cyclin G1	75.0	64.4
3 cell division cycle 20 homolog（*S. cerevisiae*）	90.2	87.9
1 apoptosis inhibitor 1（baculoviral IAP repeat-containing 3）	28.8	25.0
2 cyclin G1	75.0	64.4
3 cell division cycle 20 homolog（*S. cerevisiae*）	90.2	87.9

また，IFN はサイトカインの一種であり，免疫系に関与するサイトカインは IFN の作用と密接に関連している。インターロイキン 10(IL-10）は suppressor of cytokine signaling（SOCS）ファミリーとよばれる蛋白を発現して IFNα のシグナルを阻害することが知られており，血清中 IL-10 が高い症例は IFN 療法の効果が低いと報告されてる[8]。同様に IL-6, IL-1, TNF-α なども SOCS や，STAT の活性化を抑制する CIS を誘導することが知られており，これらのサイトカインも血清中濃度が高い症例は IFN の効果が低いと報告されている[9~11]。

● IFN 療法における単一塩基多型（SNP）

遺伝子がコードする open reading frame の中にアミノ酸変異が存在すれば，蛋白の機能に個

体差が生じることになる。また非翻訳領域においても，イントロンやプロモーターの領域に点変異，欠失や挿入などが起これば，mRNA の発現量に大きな影響を与える可能性がある。この特定の単一塩基の違いにより蛋白の質あるいは発現量に個体差が生じる現象を単一塩基多型（single nucleotide polymorphism：SNP）という。今まで述べてきた各種 IFN 関連遺伝子の発現，サイトカイン濃度などは，この SNP 解析にて遺伝子と関連付けられているものがある。

抗インフルエンザウイルス活性を示す Mx1 蛋白が高発現している症例は IFN の効果が高いとされているが，Mx 蛋白のプロモーター領域の-88 の部位に G/T の SNP が存在していることと関連している[12]。このようなプロモーター領域の SNP は IL-10[13]，細胞障害性 T 細胞の活性を抑制する CTLA4[14]，Th1 反応開始に関連する osteopontin[15]などでも認められている。SNP 解析により，IFN 療法に関連した遺伝子の発現量に差がある原因が求められ，同じウイルス量，同じ HCV ジェノタイプでも IFN 療法に差が出る背景であると考えられる。

● 包括的遺伝子情報解析

上述のように IFN 関連遺伝子，サイトカインなどのそれぞれの単一の遺伝子の発現量，あるいは蛋白の発現量により IFN 療法の効果が規定されている可能性が示唆されている。しかしながら，このような単一の遺伝子発現量のみを調べることでは，ウイルス側因子を超える IFN 療法効果予測はできない。これは，それぞれ報告されている遺伝子のみならず，未知の遺伝子を含め IFN 療法に一見関連しないと思われる多数の遺伝子が，実際には複雑に関係し合っているためと考えられる。

われわれは serial analysis of gene expression 法を用いて，正常肝を含むさまざまな肝病態における遺伝子発現プロファイルを検討し，正常肝，慢性 C 型肝炎，肝細胞癌において，それぞれ異なった遺伝子プロファイルを示すことを報告している[16]。また，cDNA マイクロアッセイ法を用いて，同じ慢性肝炎でも，C 型肝炎感染と B 型肝炎感染では異なった遺伝子発現を示すことも明らかにしてきた[17]。

このように包括的に肝内で発現している遺伝子を解析したデータをもとに，われわれは新たに IFN 効果を予測するために，IFN 療法と関連が報告されているものを中心に 295 遺伝子を載せた cDNA chip を作成し，IFN 療法を行った症例について IFN 投与前の肝生検サンプルで検討した。IFN 単独療法を行った 15 症例において発現遺伝子量を適当な解析アルゴリズムを設定することにより，IFN 投与中に血中 HCV RNA の消失が得られなかった症例と，IFN 投与中に HCV RNA 消失が得られた症例（著効例と再燃例をまとめた群）を，ウイルス量，HCV ジェノタイプと無関係に分けることが可能であった[18]。

さらに肝生検よりサンプル取得が容易な末梢血リンパ球中の遺伝子発現をみることで，IFN 治療効果予測が可能であるか検討を行った[19]。遺伝子発現の高低にかかわらず単一の遺伝子では治療効果予測の精度は良くても 50%であったが，**表 1** に示すように適切な遺伝子の組み合わせを SWEEP operator method で選んでいくと，投与前では topoisomerase (DNA) I, catenin, Ras-related C3 botulinum toxin substrate 2 の組み合わせで 91.0%の精度で治療効果予測が可能であった。同様に IFN 投与開始 2 週間後の末梢血リンパ球でも，投与前とは異なった 3 種類の遺伝子発現の組み合わせで精度 90.2%で効果予測が可能であった。

● おわりに

本稿では述べなかったが，IFN 療法のみならず C 型肝炎の病態，進行度に関しても SNP をはじめとするさまざまな遺伝子情報が蓄積されてきている。現在は研究の域を出ていないが，大量，迅速かつ安価に遺伝子情報が解析されるようになれば，個々の患者に合わせたテーラーメイド治療の時代がくるものと期待される。

文献

1) Gao B, Hong F, Radaeva S. Host factors and failure of

interferon-alpha treatment in hepatitis C virus. Hepatology 2004 ; 39 : 880-90.
2) Yatsuhashi H, Yamasaki K, Aritomi T, et al. Quantitative analysis of interferon alpha/beta receptor mRNA in the liver of patients with chronic hepatitis C : correlation with serum hepatitis C virus-RNA levels and response to treatment with interferon. J Gastroenterol Hepatol 1997 ; 12 : 460-7.
3) Mizukoshi E, Kaneko S, Yanagi M, et al. Expression of interferon alpha/beta receptor in the liver of chronic hepatitis C patients. J Med Virol 1998 ; 56 : 217-23.
4) Yatsuhashi H, Fujino T, Matsumoto T, et al. Immunohistochemical analysis of hepatic interferon alpha-beta receptor level : relationship between receptor expression and response to interferon therapy in patients with chronic hepatitis C. J Hepatol 1999 ; 30 : 995-1003.
5) Mizukoshi E, Kaneko S, Kaji K, et al. Serum levels of soluble interferon Alfa/Beta receptor as an inhibitory factor of interferon in the patients with chronic hepatitis C. Hepatology 1999 ; 30 : 1325-31.
6) Pfeffer LM, Basu L, Pfeffer SR, et al. The short form of the interferon alpha/beta receptor chain 2 acts as a dominant negative for type I interferon action. J Biol Chem 1997 ; 272 : 11002-5.
7) 八橋弘. IFN レセプター. 肝胆膵 2002 ; 45 : 981-7.
8) Kuzushita N, Hayashi N, Katayama K, et al. High levels of serum interleukin-10 are associated with a poor response to interferon treatment in patients with chronic hepatitis C. Scand J Gastroenterol 1997 ; 32 : 169-74.
9) Larrea E, Garcia N, Qian C, et al. Tumor necrosis factor alpha gene expression and the response to interferon in chronic hepatitis C. Hepatology 1996 ; 23 : 210-7.
10) Soardo G, Pirisi M, Fonda M, et al. Changes in blood lipid composition and response to interferon treatment in chronic hepatitis C. J Interferon Cytokine Res 1995 ; 15 : 705-12.
11) Kishihara Y, Hayashi J, Yoshimura E, et al. IL-1 beta and TNF-alpha produced by peripheral blood mononuclear cells before and during interferon therapy in patients with chronic hepatitis C. Dig Dis Sci 1996 ; 41 : 315-21.
12) Hijikata M, Ohta Y, Mishiro S. Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt-88) correlated with the response of hepatitis C patients to interferon. Intervirology 2000 ; 43 : 124-7.
13) Yee LJ, Tang J, Gibson AW, et al. Interleukin 10 polymorphisms as predictors of sustained response in antiviral therapy for chronic hepatitis C infection. Hepatology 2001 ; 33 : 708-12.
14) Yee LJ, Perez KA, Tang J, et al. Association of CTLA4 polymorphisms with sustained response to interferon and ribavirin therapy for chronic hepatitis C virus infection. J Infect Dis 2003 ; 187 : 1264-71.
15) Naito M, Matsui A, Inao M, et al. SNPs in the promoter region of the osteopontin gene as a marker predicting the efficacy of interferon-based therapies in patients with chronic hepatitis C. J Gastroenterol 2005 ; 40 : 381-8.
16) Yamashita T, Kaneko S, Hashimoto S, et al. Serial analysis of gene expression in chronic hepatitis C and hepatocellular carcinoma. Biochem Biophys Res Commun 2001 ; 282 : 647-54.
17) Honda M, Kaneko S, Kawai H, et al. Differential gene expression between chronic hepatitis B and C hepatic lesion. Gastroenterology 2001 ; 120 : 955-66.
18) Daiba A, Inaba N, Ando S, et al. A low-density cDNA microarray with a unique reference RNA : pattern recognition analysis for IFN efficacy prediction to HCV as a model. Biochem Biophys Res Commun 2004 ; 315 : 1088-96.
19) Tateno M, Honda M, Kawamura T, et al. Expression profiling of peripheral-blood mononuclear cells from patients with chronic hepatitis C undergoing interferon therapy. J Infect Dis 2007 ; 195 : 255-67.

Clin Exp Nephrol (2008) 12:53–57
DOI 10.1007/s10157-007-0005-6

ORIGINAL ARTICLE

Patterns in the prevalence of hepatitis C virus infection at the start of hemodialysis in Japan

Yuko Iwasa · Shigeru Otsubo · Orie Sugi · Keitaro Sato · Yukari Asamiya · Aya Eguchi · Tomihito Iwasaki · Nami Matsuda · Kan Kikuchi · Norisato Ikebe · Naoko Miwa · Naoki Kimata · Keiko Uchida · Shigeharu Uchida · Kosaku Nitta · Takashi Akiba

Received: 15 March 2007 / Accepted: 6 September 2007 / Published online: 5 January 2008

Abstract
Background Although hepatitis C virus (HCV) infection is a persistent public health concern in hemodialysis patients, there seem to have been only a few reports on the prevalence of HCV at the start of hemodialysis. In this study we investigated whether patients starting on hemodialysis therapy are positive for anti-HCV antibody or not.
Methods The 400 patients who began regular hemodialysis between February 2003 and June 2007 were enrolled in this study. Clinical data such as age, anti-HCV antibody and primary cause of end-stage kidney disease (ESKD) were examined. As healthy controls we used 70,717 healthy blood donors in 2005 whose data were obtained from Tokyo Metropolitan Red Cross Blood Center. Anti-HCV antibody was used as an indicator of HCV infection. Since the prevalence of HCV infection is affected by age in Japan, we classified the patients by age group.
Results The anti-HCV antibody prevalence rate among the patients who were new to hemodialysis was 7.3%, as opposed to 0.15% in the healthy volunteers. The prevalence of HCV in the 31–45-, 46–60-, and 61-year-old groups was significantly higher among the hemodialysis patients than among the healthy volunteers ($P = 0.0209$, <0.0001, and <0.0001, respectively). The prevalence rate of anti-HCV antibody was higher among men (10.0%) than among women (1.5%, $P < 0.0001$) in the hemodialysis patients. The anti-HCV-antibody-positive patients were significantly older than the anti-HCV-antibody-negative patients (66.4 ± 14.3 years versus 58.6±16.6 years; $P = 0.0152$). Diabetic nephropathy was a more frequent cause of ESKD among the anti-HCV-antibody-positive patients (30.4%) than among the anti-HCV-antibody-negative patients (19.9%, $P = 0.0122$). Among the anti-HCV-antibody-positive patients, 55.2% had received a blood transfusion. The rate was significantly higher than that among the anti-HCV-antibody-negative patients (19.4%, $P < 0.0001$).
Conclusion The results showed a much higher rate of anti-HCV antibody positivity in patients new to hemodialysis than in healthy volunteers. Older age, blood transfusion, male gender, and diabetic nephropathy seemed to be risk factors for anti-HCV antibody positivity in Japan.

Keywords Hepatitis C · Hemodialysis · Diabetic nephropathy · Diabetes mellitus · End-stage kidney disease (ESKD) · Liver chirrhosis

Y. Iwasa · S. Otsubo (✉) · O. Sugi · K. Sato · Y. Asamiya · A. Eguchi · N. Matsuda · K. Uchida · K. Nitta
Department of Medicine, Kidney Center,
Tokyo Women's Medical University,
8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan
e-mail: sotsubo@kc.twmu.ac.jp

T. Iwasaki · K. Kikuchi · N. Ikebe · N. Miwa · N. Kimata · T. Akiba
Department of Blood Purification, Kidney Center,
Tokyo Women's Medical University, Tokyo, Japan

S. Uchida
Tokyo Metropolitan Red Cross Blood Center, Tokyo, Japan

Introduction

Hepatitis C virus (HCV) infection is a persistent public health concern in hemodialysis patients. Unlike hepatitis B virus (HBV), no vaccine is available for HCV [1]. Patients infected with HCV often have minimal clinical evidence of disease [1, 2], but HCV infection has been associated with greater morbidity and mortality in ESKD patients [2–4].

The number of patients on hemodialysis infected with HCV is rather high [5], mostly as a result of nosocomial infection. Recent dialysis outcomes and practice patterns study (DOPPS) have revealed a mean facility prevalence in France, Germany, Italy, Japan, Spain, the UK, and the US of 13.5% and the mean prevalence according to country ranged from 2.6 to 22.9% [6]. The main causes of nosocomial infection by HCV in hemodialysis patients are filter reuse, use of contamined hemodialysis machines, and contamination of medical staff's hands [7].

Nevertheless, there seem to have been only a few reports on the prevalence of HCV at the start of hemodialysis. Some ESKD patients may be at risk of exposure to HCV associated with medical treatment, including blood transfusion, and ESKD patients are thought to be susceptible to HCV infection because of the decline in immune response. Hepatitis C is both a cause and a complication of chronic kidney disease. Chronic infection with HCV can lead to the immune complex syndromes of cryoglobulinemia and membranoproliferative glomerulonephritis (MPGN). Management of HCV-related cryoglobulinemia and MPGN is difficult: antiviral therapy is effective in clearing HCV infection in a proportion of patients, but these conditions can be severe and resistant to antiviral therapy [8]. Glomerular abnormalities in liver cirrhosis patients are also known, even though their renal insufficiency is generally mild.

An overview of regular dialysis treatment in Japan revealed that the proportion of patients who had been on hemodialysis therapy for less than 2 years who were positive for anti-HCV antibody was 7.6% [9], a higher rate than in the general population in Japan.

We therefore hypothesized that HCV infection is already relatively widespread at the start of hemodialysis therapy, and in the present study, we investigated whether patients who start hemodialysis therapy are already anti-HCV-antibody-positive.

Materials and methods

The 400 patients who started on regular hemodialysis in our kidney center at Tokyo Women's Medical University Hospital between February 2003 and June 2007 were enrolled in this study.

Age, gender, HBs antigen (Ag), HBs antibody (Ab), treponema pallidum latex immuno assay (TPLA), and primary cause of ESKD were examined. The proportions of patients starting on hemodialysis after having been on continous ambulatory peritoneal dialysis (CAPD) or having received a renal transplant were also examined. The blood chemistry, peripheral blood count and whether they had signs of liver fibrosis or hepatocellular carcinoma on abdominal echo examinations or had ever received a blood transfusion were examined.

Table 1 Prevalence of HCV in patients new to hemodialysis therapy

	Ant-HCV Ab positive	Ant-HCV Ab negative	*P* value
Number	29	371	–
Age[a] (years)	66.4 ± 14.3	58.6 ± 16.6	0.0152
Gender (M/F)	27/2	242/129	<0.0001
CAPD[b] (%)	0	3.2	n.s.
Transplantation[b] (%)	10.3	7.8	n.s.
Positive for HBs Ag (%)	0	1.08	n.s.
Positive for HBs Ab (%)	34.8	19.1	n.s.
Positive for TPLA (%)	7.1	1.64	n.s.

CAPD continuous ambulatory peritoneal dialysis, *Ag* antigen, *Ab* antibody

TPLA treponema pallidum latex immuno assay mean ± SD

[a] Age at the start of hemodialysis therapy

[b] Rate of patients switching to hemodialysis from CAPD or transplantation

As healthy controls for the prevalence of HCV we used 70,717 healthy first-time blood donors in 2005 whose data were obtained from Tokyo Metropolitan Red Cross Blood Center [10]. Since the prevalence of HCV infection is affected by age, we classified the patients into the following age groups (years): under 31, 31–45, 46–60, and 61–70.

Data are reported as means ± SD. The chi-square (χ^2) test was used for comparisons between categorical variables. Fisher's exact test was used when the criteria for the χ^2 test could not be applied. Student's *t*-test was used for comparisons between continuous variables. All statistical calculations were performed with Stat View J 5.0 software. A *P* value of less than 0.05 was considered statistically significant.

Results

The overall anti-HCV antibody prevalence rate among patients new to hemodialysis was 7.3%. Table 1 compares the anti-HCV-antibody-positive and anti-HCV-antibody-negative patients. The anti-HCV-antibody-positive patients were significantly older than the anti-HCV-antibody-negative patients (66.4 ± 14.3 years versus 58.6 ± 16.6 years; $P = 0.0152$). The prevalence rate of anti-HCV antibody was higher among men (10.0%) than among women (1.5%, $P < 0.0001$) in the hemodialysis patients. The proportions of patients starting on hemodialysis after having been on CAPD or having received a transplant were similar in both patients, and the prevalence of HBs Ag or HBs Ab was also similar in both patients. The proportion

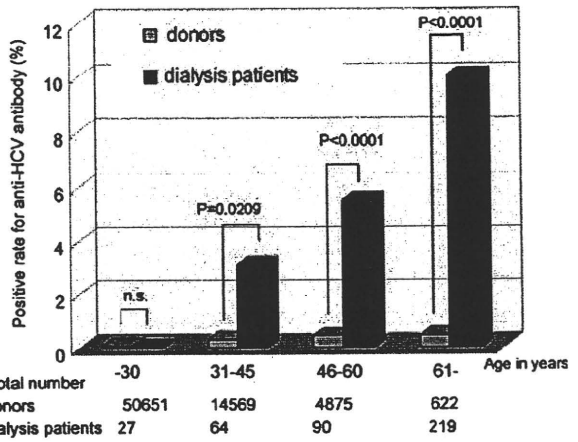

Fig. 1 Comparison between the prevalence of HCV in blood donors in Tokyo in 2005 and a new hemodialysis population in whole gender. Data on the prevalence in blood donors in Tokyo were obtained from the Tokyo Metropolitan Red Cross Blood Center

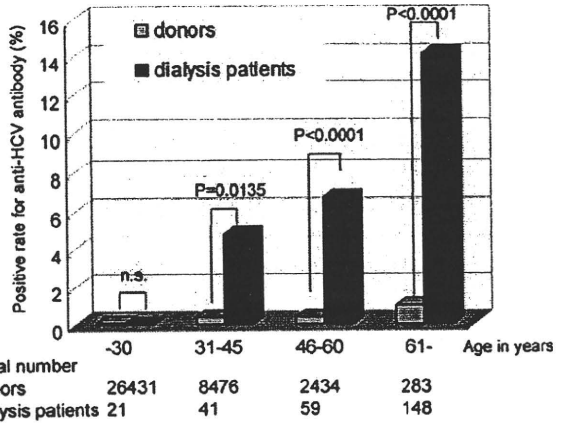

Fig. 2 Comparison between the prevalence of HCV in blood donors in Tokyo in 2005 and a new hemodialysis population in men. Data on the prevalence in blood donors in Tokyo were obtained from the Tokyo Metropolitan Red Cross Blood Center

positive for TPLA tended to be higher in anti-HCV-antibody-positive patients than in the anti-HCV-antibody-negative patients, but the difference was not statistically significant.

The anti-HCV antibody prevalence rate among the 70,717 blood donors in Tokyo in 2005 was 0.15%. Figure 1 compares the prevalence of HCV in the blood donors and the patients new to hemodialysis therapy. None of the patients new to hemodialysis in the under 31-year-old group were positive for anti-HCV antibody, whereas in the 31–45-, 46–60-, and 61-year-old groups the prevalence of HCV was significantly higher in the hemodialysis patients than in the healthy volunteers ($P = 0.0209$, <0.0001, <0.0001, respectively).

Among the 37,624 healthy male volunteers, 72 (0.19%) were positive for anti-HCV antibody, while among the 33,093 healthy female volunteers, 36 (0.11%) were positive. Similar to the trend among hemodialysis patients, the prevalence rate of anti-HCV antibody was also significantly higher among healthy male volunteers than among healthy female volunteers ($P = 0.005$). As only two women were positive for anti-HCV antibody among the hemodialysis patients, we could not compare the difference in HCV prevalence between female hemodialysis patients and female healthy volunteers. Figure 2 compares the prevalence of HCV among the blood donors and patients new to hemodialysis therapy among men. The result was similar to the overall trend for both genders.

Table 2 shows the primary causes of ESKD. Diabetic nephropathy was a more frequent cause of ESKD in the anti-HCV-antibody-positive patients (37.9%) than in the anti-HCV-antibody-negative patients (18.6%, $P = 0.0122$).

Table 3 shows the blood chemistry results, peripheral blood count, results of abdominal echography, and the history of blood transfusion for the anti-HCV-antibody-positive and anti-HCV-antibody-negative patients new to hemodialysis therapy. The total bilirubin and asparate aminotransferase levels were statistically higher among anti-HCV-antibody-positive patients (0.4 ± 0.4 mg/dl and 28.9 ± 23.3 IU/l, respectively) than among anti-HCV-antibody-negative patients (0.3 ± 0.2 mg/dl; $P = 0.0012$,

Table 2 Primary cause of end-stage kidney disease

Cause of ESKD (%)	Ant-HCV antibody positive	Ant-HCV antibody negative	*P* value
Chronic glomerulonephritis	6 (20.7%)	150 (40.4%)	n.s.
Chronic pyelonephritis	0 (0%)	1 (0.3%)	n.s.
RPGN	1 (3.4%)	9 (2.4%)	n.s.
Nephropathy of toxemia of pregnancy	0 (0%)	3 (0.8%)	n.s.
Other unclassified nephritis	1 (3.4%)	6 (1.6%)	n.s.
Polycystic kidney disease	1 (3.4%)	14 (3.8%)	n.s.
Nephrosclerosis	1 (3.4%)	51 (13.7%)	n.s.
Diabetic nephropathy	11	(37.9%)	69
(18.6%)	0.0122		
Lupus nephritis	2 (6.9%)	4 (1.1%)	n.s.
Urate nephropathy	0 (0%)	2 (0.5%)	n.s.
Urolithiasis	0 (0%)	3 (0.8%)	n.s.
Tumor of kidney or urinary tract	1 (3.4%)	3 (0.8%)	n.s.
Obstructive uropathy	0 (0%)	1 (0.3%)	n.s.
Myeloma kidney	0 (0%)	2 (0.5%)	n.s.
Renal dysplasia	0 (0%)	2 (0.5%)	n.s.
Unknown	0 (0%)	8 (2.2%)	n.s.
After renal transplantation	3 (10.3%)	29 (7.8%)	n.s.
Others	2 (6.9%)	14 (3.8%)	n.s.

RPGN rapidly progressive glomerulonephritis

Table 3 Characteristics of patients new to hemodialysis therapy

Category	Ant-HCV antibody positive	Ant-HCV antibody negative	*P* value
Total bilirubin (mg/dl)	0.4 ± 0.4	0.3 ± 0.2	0.0012
Asparate aminotransferase (IU/l)	28.9 ± 23.2	18.6 ± 16.7	0.0022
Alanine aminotransferase (IU/l)	25.2 ± 17.4	17.3 ± 25.5	n.s.
Fe (μg/dl)	58.4 ± 60.8	59.4 ± 35.3	n.s.
Total iron binding capacity (μg/dl)	222 ± 58	227 ± 51	n.s.
Ferritin (ng/ml)	354 ± 273	305 ± 472	n.s.
White blood cell count (/μl)	6,400 ± 3,240	6,750 ± 2,720	n.s.
Red blood cell count ($\times 10^6$/μl)	2.78 ± 0.58	2.85 ± 0.54	n.s.
Hemoglobin (g/dl)	8.4 ± 1.4	8.6 ± 1.6	n.s.
Hematocrit (%)	25.5 ± 4.6	26.2 ± 4.9	n.s.
Platelet count ($\times 10^4$/μl)	17.0 ± 7.1	19.8 ± 8.2	n.s.
Liver fibrosis (%)	25.0	4.9	0.0002
Hepatocellular carcinoma (%)	17.9	1.4	<0.0001
Blood transfusion (%)	55.2	19.4	<0.0001

Mean ± SD

18.6 ± 16.7 IU/l; $P = 0.0022$). The alanine aminotransferase level tended to be higher among the anti-HCV-antibody-positive patients (25.2 ± 17.4 IU/l) than among the anti-HCV-antibody-negative patients (17.3 ± 25.5 IU/l). Iron-related markers like the Fe level, the total iron-binding capacity, and the ferritin level were almost the same among anti-HCV-antibody-positive and anti-HCV-antibody-negative patients. Similarly, the white blood cell count, hemoglobin and hematocrit levels were almost the same, but the platelet count tended to be lower among the ant-HCV-antibody-positive patients ($17.0 \pm 7.1 \times 10^4$/μl) than among the anti-HCV-antibody-negative patients ($19.8 \pm 8.2 \times 10^4$/μl). The rates of liver fibrosis and hepatocellular carcinoma were statistically higher among anti-HCV-antibody-positive patients (25.0% and 17.9%) than among anti-HCV-antibody-negative patients (4.9%; $P = 0.0002$, 1.4%; $P < 0.0001$). Among the anti-HCV-antibody-positive patients, 55.2% had received a blood transfusion. This rate was significantly higher than that among the anti-HCV-antibody-negative patients (19.4%, $P < 0.0001$).

Discussion

The prevalence of HCV infection at the start of hemodialysis therapy has never been clearly described in Japan. A study in Italy reported an anti-HCV-antibody-positive rate at the start of hemodialysis therapy of 13% [11]. An anti-HCV prevalence rate of 14.4% at the start of hemodialysis therapy was reported by a study in the US, and age, race, gender, and drug abuse were all independent predictors of anti-HCV antibody positivity in that study population [12]. The US study reported that age (50>) was a significant predictor, that younger patients were more likely to be infected with HCV, and that black men and former or current drug abusers were more likely to test positive for anti-HCV antibody. Presumably, such high-risk behaviors as drug abuse are more common among younger patients, men, and blacks, thereby contributing to the high frequency of HCV infection in incident dialysis patients belonging to these patient groups [12]. The prevalence of anti-HCV antibody among patients new to hemodialysis in our study was 7.3%. As Fig. 1 shows, the prevalence of anti-HCV antibody was significantly higher among patients new to hemodialysis than among the healthy controls in subjects over the age of 31 years. Similar to the results of a study conducted in the US, male gender was a risk factor for anti-HCV-antibody positivity in this study. On the other hand, the anti-HCV-antibody-positive patients were older than the anti-HCV-antibody-negative patients at the start of hemodialysis, and the rate of patients who had received a blood transfusion was higher among the anti-HCV-antibody-positive patients than among the anti-HCV-antibody-negative patients. In contrast to the US, older age and blood transfusion may be risk factors in Japanese patients. Donor blood was not routinely screened for HCV infection in Japan until 1989. Older patients may have received unscreened blood that transmitted HCV infections.

HCV infection may cause ESKD, but none of the patients had ever undergone a renal biopsy and been diagnosed with HCV-related glomerulonephritis. The frequent presence of glomerular abnormalities in patients with liver cirrhosis was first noted during the 1940s. The renal insufficiency is generally mild in such patients. Twenty-five percent of patients positive for anti-HCV antibody already had liver fibrosis. The total bilirubin and asparate aminotransferase levels were statistically higher among the anti-HCV-antibody-positive patients than among the anti-HCV-antibody-negative patients. The alanine aminotransferase level tended to be higher, and platelet count tended to be lower among the anti-HCV-antibody-positive patients than among the anti-HCV-antibody-negative patients. Renal dysfunction secondary to liver fibrosis may have affected their renal survival even though the primary cause of the ESKD was something else, for example diabetic nephropathy.

Our hospital has two hemodialysis rooms, one in the kidney center and the other in the diabetes center. The subjects of this study were patients who started

hemodialysis in the kidney center, so the percentage of patients who started on hemodialysis for ESKD secondary to diabetic nephropathy was relatively low.

A high prevalence of HCV has been reported in patients with type-two diabetes mellitus (DM) [13]. The high prevalence may be related to increased vulnerability to HCV infection because of impaired immune defence mechanisms in DM. Also, some patients with various forms of liver disease are predisposed to impaired glucose tolerance because of corticosteroid and hydrochlorothiazide therapy or the presence of hemochromatosis [14]. In addition to these known risk factors, there are emerging epidemiological data suggesting that HCV infection may also contribute to the development of diabetes [15]. Two reports have mentioned the high prevalence of HCV infection among hemodialysis patients with diabetes mellitus [13, 16]. Our study revealed that diabetic nephropathy was a more frequent cause of ESKD among Japanese patients who were anti-HCV-antibody positive at the start of hemodialysis (37.9%) than among those who were anti-HCV-antibody negative (18.6%, $P = 0.0122$).

The prevalence of HCV was not very high among the patients starting on hemodialysis after having been on CAPD or who had received a transplant. Previous CAPD or transplantation was not a risk factor for HCV infection in this study.

Low levels of iron and ferritin are advantageous for the activity of hepatitis because of the reduced reactive oxidative stress. However, no differences in the iron and ferritin levels were observed between the anti-HCV-antibody-positive and the anti-antibody-negative patients.

One of the limitations of this study is that Tokyo Metropolitan Red Cross Blood Center accepts volunteers who do not have history of blood transfusion, history of viral hepatitis, or other risk factors as blood donors. So the volunteer blood donors, even first time ones, have been documented to have lower infection rates than the general population.

An overview of regular dialysis treatment in Japan reported a 7.6% anti-HCV antibody-positive rate among patients who had been on hemodialysis therapy for less than 2 years [9], and there were no statistical differences between the patients with less than 2 years hemodialysis in their study and the patients new to hemodialysis in our study. Acquisition of hepatitis C from nosocomial sources after starting on dialysis therapy appears to be much less of a factor now.

Conclusion

The results of this study showed a much higher rate of anti-HCV antibody positivity in patients new to hemodialysis than in healthy volunteers. Older age, blood transfusion, male gender, and diabetic nephropathy seemed to be risk factors for anti-HCV antibody positivity in Japan.

References

1. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med. 2001;345:41–52.
2. Fabrizi F, Poordad FF, Martin P. Hepatitis C infection and the patient with end-stage renal disease. Hepatology. 2002;36:3–10.
3. Nakayama E, Akiba T, Marumo F, Sato C. Prognosis of anti-hepatitis C virus antibody-positive patients on regular hemodialysis therapy. J Am Soc Nephrol. 2000;11:1896–1902.
4. Maisonneuve P, Agodoal L, Gellert R et al Cancer in patients on dialysis for end-stage renal disease: an international collaborative study. Lancet. 1999;354:93–9.
5. Kikuchi K, Yoshida T, Kimata N, Sato C, Akiba T. Prevalence of hepatitis E virus infection in regular hemodialysis patients. Ther Apher Dial. 2006;10:193–7.
6. Fissell RB, Bragg-Gresham JL, Woods JD, Jadoul M, Gillespie B, Hedderwick SA, Rayner HC, Greenwood RN, Akiba T, Young E. Patterns of hepatitis C prevalence and seroconversion in hemodialysis units from three continents: The DOPPS. Kidney Int. 2004;65:2335–42.
7. Fabrizi F, Poordad FF, Martin P. Hepatitis C infection and the patient with end-stage renal disease. Hepatology. 2002;36:3–10.
8. Meyers CM, Seeff LB, Stehman-Breen CO, Hoofnagle JH. Hepatitis C and renal disease: an update. Am J Kidney Dis. 2003;42:631–57.
9. An overview of regular dialysis treatment in Japan as of December 31, 2006. Japanese society for dialysis therapy: 2007.
10. Tokyo Metropolitan Blood Center. 2006 annual report of Japanese Red Cross Tokyo Metropolitan Blood Center: Tokyo, Japan, 2006; 1–6.
11. Petrosillo N, Gilli P, Serraino D, Dentico P, Mele A, Ragni P, Puro V, Casalino C, Ippolito G. Prevalence of infected patients and understaffing have a role in hepatitis C virus transmission in dialysis. Am J Kidney Dis. 2001;37:1004–10.
12. Bergman S, Accortt N, Turner A, Glaze J. Hepatitis C infection is acquired pre-ESRD. Am J Kidney Dis. 2005;45:684–9.
13. Ocak S, Duran N, Kaya H, Emir I. Seroprevalence of hepatitis C in patients with type 2 diabetes mellitus and non-diabetic on haemodialysis. Int J Clin Pract. 2006;60:670–4.
14. Petrides AS. Liver disease and diabetes mellitus. Diabetes Rev. 1994;2:2–18.
15. Mason AL, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, Guo L, Jacob S, Regenstein FG, Zimmerman R, Everhart JE, Wasserfall C, Maclaren NK, Perrillo RP. Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology. 1999;29:328–33.
16. Saxena AK, Panhotra BR. The susceptibility of patients with type-2 diabetes to hepatitis C virus infection during long-term haemodialysis. Swiss Med Wkly. 2003;133:611–8.

Jpn. J. Infect. Dis., 61, 2008

Laboratory and Epidemiology Communications

Positivity Rate of Hepatitis B Surface Antigen in 16-Year-Old First-Time Blood Donors: Effectiveness of Immunoprophylaxis with Hepatitis B Vaccine and Immunoglobulin in Newborn Infants with Mothers Positive for Hepatitis B e Antigen

Shigeharu Uchida* and Kenji Tadokoro[1]

Tokyo Western Red Cross Blood Center, Tokyo 190-0014, and
[1]Japanese Red Cross Central Blood Institute, Tokyo 135-8521, Japan

Communicated by Takaji Wakita

(Accepted November 30, 2007)

In 1986, a program to prevent mother-to-infant transmission of hepatitis B virus (HBV) was initiated in Japan. Since that time, expecting mothers have been routinely tested for hepatitis B surface antigen (HBsAg). When a mother is found to be HBsAg-positive, she is also tested for hepatitis B e antigen (HBeAg). If a mother tests positive for both HBsAg and HBeAg, the newborn infant is administered the hepatitis B vaccine and immunoglobulin at the public's expense. The rate of HBsAg testing of expecting mothers was calculated to be 92-96%, as determined by comparison of the number of expecting mothers tested for HBsAg and the number of births during the following year (1). Moreover, 97-98% of newborn infants with HBeAg-positive mothers were administered the hepatitis B vaccine together with immunoglobulin (1). This mother-to-infant HBV transmission prevention program was expanded in March of 1995 to include not only newborn infants with HBeAg-positive mothers, but also infants with HBeAg-negative mothers.

Since 1995, HBsAg-positivity rate of first-time blood donors in Tokyo, Ibaraki, Tochigi, Kanagawa, and Fukuoka Prefectures has been investigated. Because first-time blood donors are unaffected by prior notification of previous screening test results, the positivity rate of first-time blood donors is thought to reflect the positivity rate of the community in general. To evaluate the effectiveness of the mother-to-infant HBV transmission prevention program, we compared the HBsAg positivity rate of 16-year-old first-time blood donors before and after 2003, because all 16-year-old blood donors in 2003 in these areas were born in the same year, 1986.

The HBsAg positivity rate of 16-year-old first-time blood donors was found to decline yearly starting in 1995, and finally reached zero in 2003 (Fig. 1). In 2004, the positivity rate increased, but it was confirmed that all HBsAg-positive persons identified in that year had been infected via a horizontal transmission route. Because the HBsAg-positivity rate in 2005 returned to zero, no HBV carrier was identified among 16-year-old first-time blood donors for the 3 years from 2003 to 2005. However, one HBV carrier was confirmed in 2006. Investigation revealed that this case was due to a failure of immunoprophylaxis.

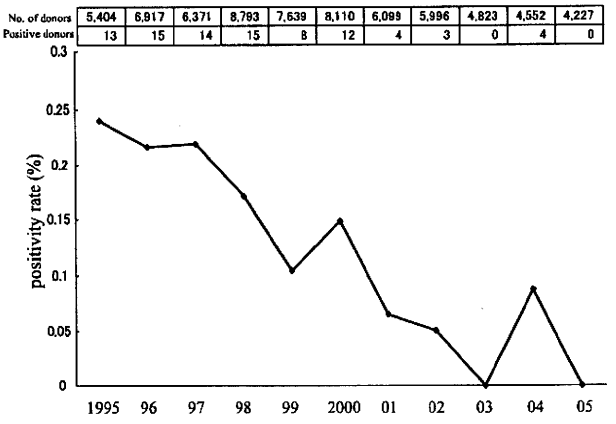

Fig. 1. Positivity rate of HBsAg in 16-year-old first-time blood donors. The investigation was performed in Tokyo, Ibaraki, Tochigi, Kanagawa, and Fukuoka Prefectures.

The effectiveness of this program to prevent mother-to-infant transmission of HBV was confirmed by investigation of the HBsAg-positivity rate of 16-year-old first-time blood donors. It is expected that the continuity of this prevention program will substantially reduce the number of HBV carriers in Japan.

The HBsAg-positivity rate of 16-year-old first-time blood donors was found to decline yearly after the onset of this study in 1995. This decline may be attributed to the following factors: (i) clinical immunoprophylaxis trials using hepatitis B vaccine and immunoglobulin had been carried out prior to the initiation of the national mother-to-infant HBV transmission prevention program, and (ii) the number of infants with HBeAg-positive mothers decreased due to late marriage.

REFERENCE

1. Shiraki, K. (1994): Vertical transmission of hepatitis B virus and its prevention in Japan. p. 530-532. *In* Viral Hepatitis and Liver Disease. Springer-Verlag, Tokyo.

*Corresponding author: Mailing address: Tokyo Western Red Cross Blood Center, 3256 Midori-cho, Tachikawa-City, Tokyo 190-0014, Japan. Tel: +81-42-529-0401, Fax: +81-42-529-0402, E-mail: shi-uchida@tokyo.bc.jrc.or.jp

Transfusion Medicine, 2009, **19**, 172–179 doi: 10.1111/j.1365-3148.2009.00930.x

ORIGINAL ARTICLE

Effect of selective vaccination on a decrease in the rate of hepatitis B virus-positive Japanese first-time blood donors

A. Yoshikawa,* K. Suzuki,† A. Abe,‡ T. Tanaka,† K. Yamaguchi,§ T. Tanaka,¶ Y. Ishikawa,† K. Minegishi,† Y. Gotanda,* H. Yugi,** S. Uchida,† M. Satake,** H. Mizoguchi* & K. Tadokoro†

**Japanese Red Cross Saitama Blood Center, 1370-12 Takahagi, Hidaka-shi, Saitama-ken 350-1213, †Blood Services Department, Japanese Red Cross Headquarters, 2-1-67 Tatsumi, Koutou-ku, Tokyo 135-8521, ‡ATR Knowledge Science Laboratories, 2-2-2 Hikaridai, Seika-cho, Soraku-gun, Kyoto 619-0288, §Department of Statistics, Hitotsubashi University, 2-1 Naka, Kunitachi-shi, Tokyo 186-8601, ¶International Research and Educational Institute for Integrated Medical Science, Tokyo Woman's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, **Japanese Red Cross Tokyo Nishi Blood Center, 3256 Midori-cho, Tachikawa-shi, Tokyo 190-0014, Japan*

Received 6 November 2008; accepted for publication 4 February 2009

SUMMARY. The government of Japan started a selective vaccination programme to prevent mother-to-infant infection by hepatitis B virus (HBV) since January 1986. The effect of the programme on first-time blood donors has not been examined in detail. Data of first-time blood donors aged 16–25 years from 1996 to 2007 were extracted from the Japanese Red Cross (JRC) donors' database. Principal component analysis (PCA) was used to visualize the birth-year-dependent group of rate of HBV-positive donors. According to the birth of year, donors were divided into four groups by PCA. After the start of the programme, donors born in 1986–1989 comprised a single group. Before the start of the programme, three groups (1980, 1981–1984 and 1985) were identified. Although a significant time-dependent decrease in the rate of HBV-positive donors was observed before the start of the programme, a significant difference in the rate of HBV-positive donors was observed around the start of the programme by regression analysis for 16–19-year-old first-time blood donors. The selective vaccination programme has been effective to prevent the vertical transmission of HBV from the analysis of first-time blood donors. On the other hand, vaccination of blood donors should be considered to reduce the risk of post-transfusion HBV infection, because the horizontal transmission increases in HBV-positive blood donors.

Key words: first-time blood donors, HBV selective vaccination, principal component analysis, regression analysis.

South and East Asia including Japan was an epidemic area of hepatitis B virus (HBV). From the report of the Japanese Ministry of Health, Labour and Welfare in 2002, the number of HBV-infected patients was 97 000, and asymptomatic carriers were estimated to be 1·1–1·4 million. It is reported that the estimated number of HBV carriers was 0·63% and that of hepatitis C virus (HCV) carriers was 0·49% among Japanese first-time blood donors in 1995–2000 (Tanaka *et al.*, 2004). However, recently, HBV infection rate among Japanese first-time blood donors has been decreasing markedly. The recent rate of positive first-time blood donors for the HBV surface antigen (HBsAg) was < 0·22%.

Infection routes of HBV were divided into two main routes, the vertical (mother-to-infant) and horizontal routes. Most of the vertical infections become chronic and most of the horizontal infections end transiently.

Since January 1986, the government of Japan started a nationwide programme to prevent mother-to-infant infection by HBV (Shiraki, 1994; Shiraki *et al.*, 1996; Inui *et al.*, 2007). Every pregnant woman has been screened for serum HBsAg and the HBV e antigen

Correspondence: A. Yoshikawa, Japanese Red Cross Saitama Blood Center, 1370-12 Takahagi, Hidaka-shi, Saitama-ken 350-1213, Japan.
Tel.: +81 42 985 6111; fax: +81 42 985 6907
e-mail: yoshikawa@saitama.bc.jrc.or.jp

(HBeAg). Newborn infants whose mothers were positive for HBeAg were received an immunoprophylaxis treatment by administrating a hepatitis B vaccine and hepatitis B immunoglobulin (HBIG)

The Ministry of Health and Welfare issued a notification on the use of disposable syringes in addition to disposable needles in 1988. This notification might contribute to reducing the risk of iatrogenic HBV infections when babies were administered several mandatory vaccines. With the implementation of this prevention programme, transmission of HBV decreased markedly yearly. On the other hand, horizontal infection with HBV genotype A, which seldom appeared in Japan several years ago, has increased recently in both patients and donors (Orito *et al.*, 2001; Murokawa *et al.*, 2005; Sugauchi *et al.*, 2006; Takeda *et al.*, 2006; Hayashi *et al.*, 2007).

To investigate the recent epidemiology of HBV infection and the effectiveness of the Japanese vaccination programme for the prevention of mother-to-infant transmission of HBV, the data of HBsAg-positive Japanese donors aged 16–25 years were used for principal component analysis (PCA) and regression analysis.

MATERIALS AND METHODS

The Japanese government started a nationwide hepatitis B vaccination programme in January 1986 for infants born to HBV-carrier mothers to prevent perinatal infection of HBV (Shiraki, 1994; Shiraki *et al.*, 1996; Inui *et al.*, 2007). Initially, the Japanese vaccination programme covered only neonates born to mothers who were positive for both HBsAg and the HBeAg. In 1995, the vaccination programme was extended to all neonates born to mothers who were HBsAg carriers regardless of the mother's HBeAg/antibody status. More than 92% of all the pregnant women in Japan were enrolled in the programme (Inui *et al.*, 2007).

The number of first-time blood donors and HBsAg-positive donors aged 16–25 years was extracted from JRC database from 1996 to 2007. To investigate the present state of HBV infection, the presence of the immunoglobulin-M antibody against the HBV core antigen (IgM-HBcAb) was determined among all HBsAg-positive donors from October 2006 to September 2007. The Japanese screening system was reported previously (Iizuka *et al.*, 1992; Yugi *et al.*, 2006). The nucleic acid amplification technology (NAT) system has been reported elsewhere (Mine *et al.*, 2003). IgM-HBcAb was tested by enzyme immunoassay (Abbott Laboratories, IL, USA).

Statistical analysis

The effect of the Japanese vaccination programme on the rate of HBsAg-positive donors was examined by principal component analysis (PCA) and regression analysis.

The rate of HBsAg-positive 16–18-year-old donors for every birth of year from 1980 to 1989 was visually grouped by PCA (Appendix) using the free software R (http://www.r-project.org/).

The difference in the rate of HBsAg-positive donors between before and after the implementation of the vaccination programme was analysed. We assumed the different slope around 1986 and intended to verify the assumption by regression analysis using the following equation

$$y_n = \alpha_n + \beta_n x_1 + \gamma_n x_2 + \delta_n D + \varepsilon_n, \qquad (1)$$

where α is a constant, β the coefficient of slope after 1980, γ the additional coefficient of slope after 1986, δ the coefficient (D) that shows the gap of HBsAg-positive rate around 1986, ε the error term that meets the standard assumption, n the age from 16 to 25 years old and x_1, x_2 the rate of positive donors of years born from 1980 to 1991. Regression analysis was carried out using the Microsoft Office Excel software.

RESULTS

Time-dependent changes in the rate of HBsAg-positive first-time blood donors of each generation from 1996 to 2007 are shown in Fig. 1. The rate of HBsAg-positive younger generations was lower than those of older generations. The rate of HBsAg-positive donors of all generations decreased yearly from 0·83% in 1996 to 0·22% in 2007 (data not shown).

Present state of numbers of blood donors and numbers of HBV-infected blood donors in Japan is shown in Table 1. From October 2006 to September 2007, the total number of donors was 4 974 911: the number of HBsAg-positive donors was 2043 (0·041%), the number of first-time blood donors was 594 096 and the number of HBsAg-positive first-time blood donors was 1362 (0·229%). Among 61 IgM-HBcAb-positive donors, 35 were repeat donors who have been infected after the last donation. Among 90 HBsAg-negative and NAT-positive donors, 22 were considered to have occult HBV infection on the basis of their being HBsAg-negative, HBV-DNA-positive and IgG-HBcAb-positive. Then serological window period donors were 68 among NAT-positive donors.

The rate of HBsAg-positive Japanese donors from 16 to 25 years old was extracted from JRC database from

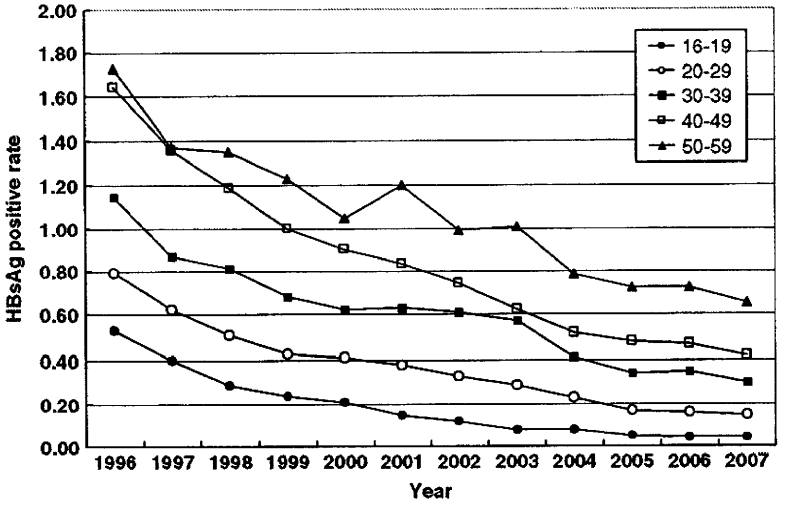

Fig. 1. Age–time-dependent rate of HBsAg-positive first-time blood donors from 1996 to 2007. Data of donors in their 60s are omitted because the number of first-time blood donors in their 60s is so small that the amplitude of the rate of HBsAg-positive donors becomes large unexpectedly.

Table 1. Present state of numbers of blood donors and numbers of HBV-infected blood donors from October 2006 to September 2007 in Japan

	Total blood donors			First-time blood donors			Horizontal infection	
Age	Number of donors	Number of HBsAg-positive donors	Rate (%) of HBsAg-positive donors	Number of donors	Number of HBsAg-positive donors	Rate (%) of HBsAg-positive donors	IgM-HBcAb-positive donors	NAT-positive donors
16	37 717	3	0·008	30 436	3	0·010	0	0
17	53 388	10	0·019	29 277	10	0·034	0	0
18	118 711	31	0·026	66 617	29	0·044	1	0
19	130 391	25	0·019	51 080	20	0·039	2(1)*	2
20	124 224	31	0·025	33 847	27	0·080	4(3)	2
21	120 609	28	0·023	25 583	22	0·086	3(2)	4
22	118 215	38	0·032	22 806	32	0·140	2(1)	1
23	118 974	38	0·032	20 640	30	0·145	3(2)	4
24	115 434	37	0·032	17 873	32	0·179	2(1)	3
25	110 247	38	0·034	15 574	30	0·193	2	4
26–29	452 645	172	0·038	50 433	130	0·258	6(3)	9
30–39	1 375 372	499	0·036	112 620	333	0·296	24(19)	25
40–49	1 077 348	487	0·045	64 232	286	0·445	9(2)	10
50–59	773 571	484	0·063	44 004	296	0·673	3(1)	15(12)†
60–69	248 065	122	0·049	9 074	82	0·904	0	11(10)
Total	4 974 911	2043	0·041	594 096	1362	0·229	61(35)	90(22)

*Number of repeated donors are shown in parenthesis.
†Number of IgG-HBcAb-positive donors (occult donors) are shown in parenthesis.

1996 to 2007 to investigate the effectiveness of the Japanese vaccination programme. The rates of HBsAg-positive first-time blood donors from 16 to 25 years old who are born from 1980 to 1991 are shown in Table 2.

The bold line between data in 1985 and those in 1986 shows the boundary before and after the implementation of the Japanese vaccination programme. The lowest column in Table 2 shows that the rate of HBsAg-positive 16-year-old donors who were born in 1991, and became acceptable as blood donors for the first time in 2007, was 0·018%.

To visualize the difference in the rate of HBsAg-positive donors around the start of the vaccination programme, PCA was carried out using the data within the frame of the dotted line in Table 2. From the result of the PCA of HBsAg-positive 16- to 18-year-old donors born from 1980 to 1989, the donors can be divided into four groups (Fig. 2).

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									

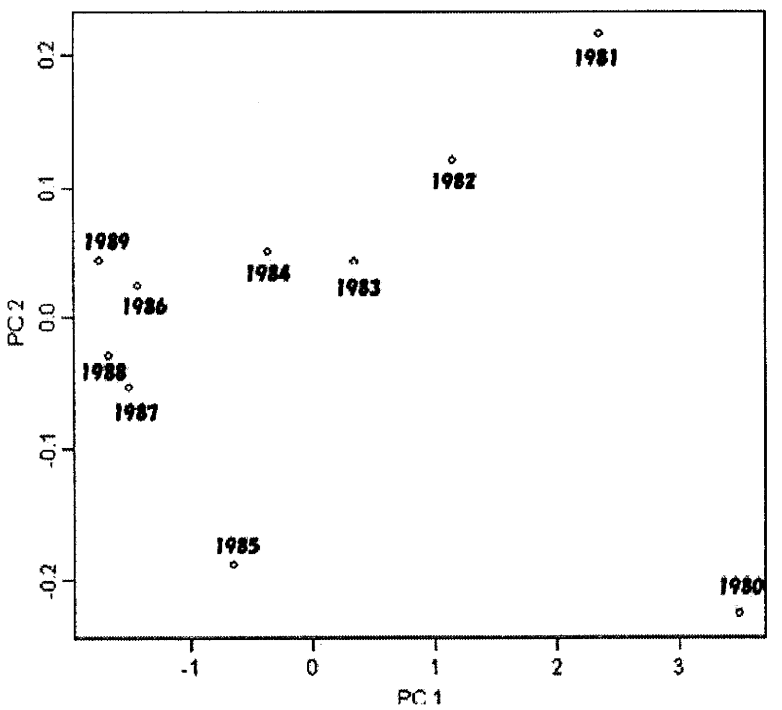

Fig. 2. Two-dimensional display of results of PCA of rate of HBsAg-positive first-time blood donors. Calculation was carried out with the free software 'R' (http://www.r-project.org/) using data within the frame of the dotted line in Table 2 (16-, 17- and 18-year-old donors born from 1980 to 1989). PCA 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.

ACKNOWLEDGMENTS

We are grateful to all members of blood centres in Japan, reporting data and sending samples. We also gratefully acknowledge the guidance and support of the President of JRC, Mr T. Konoe, the emeritus professor Dr M. Mayumi, Jichi Medical School and the former Counsellor of JRC, Dr K. Nishioka.

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) × $\sqrt{}$(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.

REFERENCES

Chiyoda, S., Oda, H., Kotani, T., Kusuba, E. & Kusano, T. (2006) The effect of a nationwide prevention program for mother-to-infant infection by HBV evaluated by viral markers in blood donors in Nagasaki prefecture. *Japan Journal of Transfusion Medicine*, **52**, 52–56.

Gervais, A., Longuet, P. & Leport, C. (2008) A success story: universal vaccination in Italy–what has to be done to eradicate residual acute hepatitis B? *Clinical Infectious Diseases*, **46**, 876–877.

Hanè, S., Ramsay, M., Balogun, K., Edmunds, W.J. & Mortimer, P. (2004) Incidence and routes of transmission of hepatitis B virus in England and Wales, 1995–2000: implications for immunization policy. *Journal of Clinical Virology*, **29,** 211–220.

Hayashi, K., Katano, Y., Takeda, Y. *et al.* (2007) Comparison of hepatitis B virus subgenotypes inpatients with acute and chronic hepatitis B and absence of lamivudine-resistant strains in acute hepatitis B in Japan. *Journal of Medical Virology*, **79,** 366–373.

Hens, N., Aerts, M., Shkedy, Z., Kung'ukimani, P., Kojouhorova, M., Vandamme, P. & Beutels, Ph. (2008) Estimating the impact of vaccination using age-time-dependent incidence rates of hepatitis B. *Epidemiology and Infection*, **136,** 341–351.

Iizuka, H., Ohmura, K., Ishijima, A. *et al.* (1992) Correlation between anti-HBc titers and HBV DNA in blood units without detectable HBsAg. *Vox Sanguinis*, **63,** 107–111.

Inui, A., Komatsu, H., Sogo, T., Nagai, T., Abe, K. & Fujisawa, T. (2007) Hepatitis B virus genotypes in children and adolescents in Japan: before and after immunization for the prevention of mother to infant transmission of hepatitis B virus. *Journal of Medical Virology*, **79,** 670–675.

Koibuchi, T., Hitani, A., Nakamura, T., Nojiri, N., Nakajima, K., Jyuji, T. & Iwamoto, A. (2001) Predominance of genotype A HBV in an HBV-HIV-1 dually positive population compared with an HIV-1-negative counterpart in Japan. *Journal of Medical Virology*, **64,** 435–440.

Mele, A., Tosti, M.E., Mariano, A. *et al.* (2008) Acute hepatitis B 14 years after the implementation of universal vaccination in Italy: areas of improvement and emerging challenges. *Clinical Infectious Diseases*, **46,** 868–875.

Mikaeloff, Y., Caridade, G., Assi, S., Tardieu, M. & Suissa, S. (2007) Hepatitis B vaccine and risk of relapse after a first childhood episode of CNS inflammatory demyelination. *Brain*, **130,** 1105–1110.

Mine, H., Emura, H., Miyamoto, M. *et al.* (2003) 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. *Journal of Virological Methods*, **112,** 145–151.

Murokawa, H., Yoshikawa, A., Ohnuma, H. *et al.* (2005) Epidemiology of blood donors in Japan, positive for hepatitis B virus and hepatitis C virus by nucleic acid amplification testing. *Vox Sanguinis*, **88,** 10–16.

Ni, Y.H., Huang, L.M., Chang, M.H. *et al.* (2007) Two decades of universal hepatitis B vaccination in Taiwan: impact and implication for future strategies. *Gastroenterology*, **132,** 1287–1293.

O'Brien, S.F., Fan, W., Xi, G. *et al.* (2008) Declining hepatitis C rates in first-time blood donors: insight from surveillance and case–control risk factor studies. *Transfusion*, **48,** 902–909.

Ohnuma, H., Yoshikawa, A., Mizoguchi, H. & Okamoto, H. (2005) Characterization of genotype H hepatitis B virus strain identified for the first time from a Japanese blood donor by nucleic acid amplification test. *The Journal of General Virology*, **86,** 595–599.

Orito, E., Ichida, T., Sakugawa, H. *et al.* (2001) Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. *Hepatology*, **34,** 590–594.

Ringwald, J., Mertz, I., Zimmermann, R. *et al.* (2005) Hepatitis B virus vaccination of blood donors–what costs may be expected? *Transfusion Medicine*, **15,** 83–92.

Shiraki, K. (1994) Vertical transmission of hepatitis B virus and its prevention in Japan. In: *Viral Hepatitis and Liver Disease* (eds Nishioka, K., Suzuki, H., Mishiro, S. & Oda, T.), 530–532. Spring-Verlag, Tokyo.

Shiraki, K., Nagata, I., Iizuka, T. & Kaji, S. (1996) Mother-to-infant infection by hepatitis B virus and its prevention in Japan. *International Hepatology Communications*, **5,** 74–78.

Su, F.H., Chen, J.D., Cheng, S.H., Sung, K.Y., Jeng, J.J. & Chu, F.Y. (2008) Waning-off effect of serum hepatitis B surface antibody amongst Taiwanese university students: 18 years post-implementation of Taiwan's national hepatitis B vaccination programme. *Journal of Viral Hepatitis*, **15,** 14–19.

Sugauchi, F., Orito, E., Ohno, T. *et al.* (2006) Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. *Hepatology Research*, **36,** 107–114.

Takeda, Y., Katano, Y., Hayashi, K. *et al.* (2006) Difference of HBV genotype distribution between acute hepatitis and chronic hepatitis in Japan. *Infection*, **34,** 201–207.

Tanaka, J., Kumagai, J., Katayama, K. *et al.* (2004) 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*, **47,** 32–40.

Uchida, S. & Tadokoro, K. (2008) Positive rate of hepatitis B surface antigen in 16-year-old first-time blood donors: effectiveness of immunoprophylaxis with hepatitis B vaccine and immunoglobulin in newborn infants with mothers positive for hepatitis B e antigen. *Japanese Journal of Infectious Diseases*, **61,** 94.

World Health Organization. (1992) Expanded programme on immunization global advisory group. *Weekly Epidemiological Record*, **3,** 11–16.

Yugi, H., Mizui, M., Tanaka, J. & Yoshizawa, H. (2006) Hepatitis B virus (HBV) screening strategy to ensure the safety of blood for transfusion through a combination of immunological testing and nucleic acid amplification testing–Japanese experience. *Journal of Clinical Virology*, **36,** S56–S64.

肝　炎

献血者におけるB型肝炎ウイルスと輸血後肝炎

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

◆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

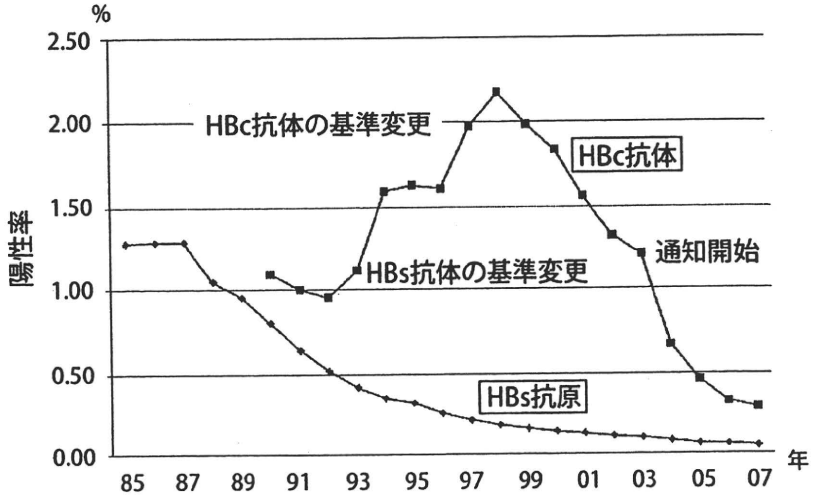

図1　献血者におけるHBs抗原・HBc抗体陽性率

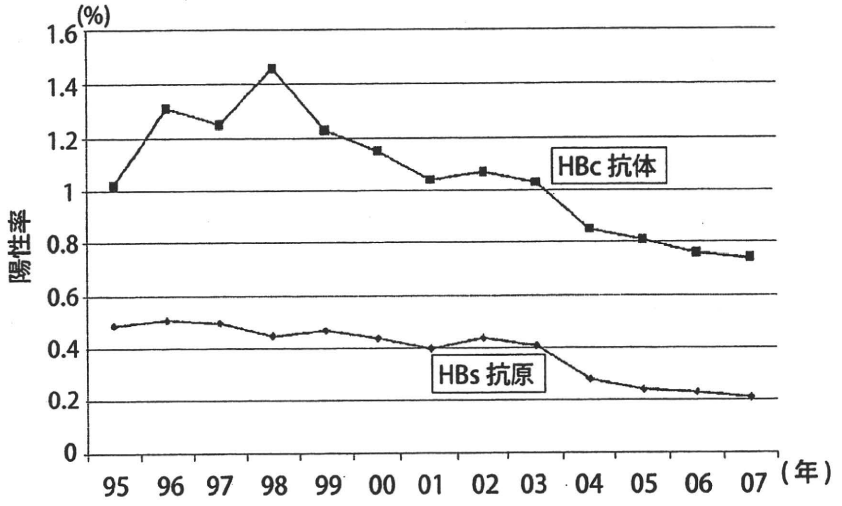

図 2　初回献血者における HBs 抗原・HBc 抗体陽性率

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

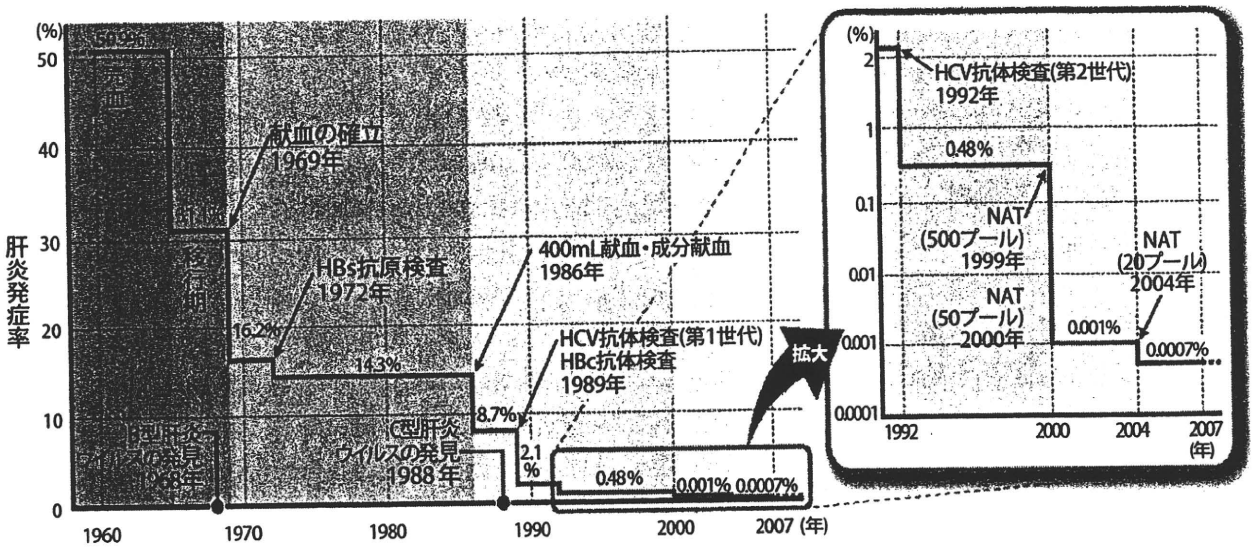

図3　我が国における輸血後肝炎の推移（『輸血情報 0811-116』）

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 NAT	1997	12	1
	1998	22	7
	1999	21	5
	2000	5	0
	2001	7	0
	2002	8	0
after NAT	2003	12	0
	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