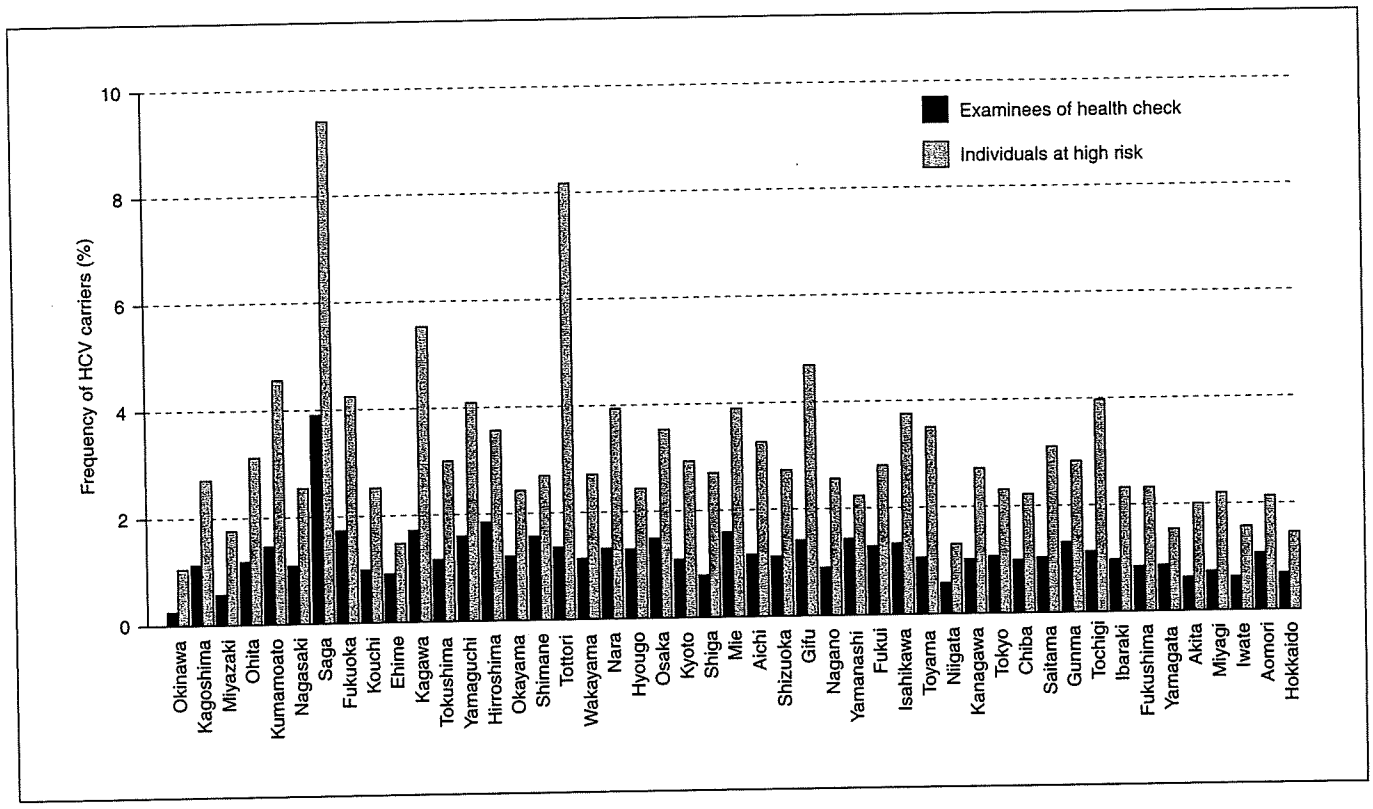


Fig. 9. Distribution of persistent HCV infection in examinees at regular health check-ups and individuals at high risk from the 48 jurisdictions in Japan detected by the national screening during the first fiscal year. The 48 jurisdictions are ordered from southeast on the left to northeast on the right.

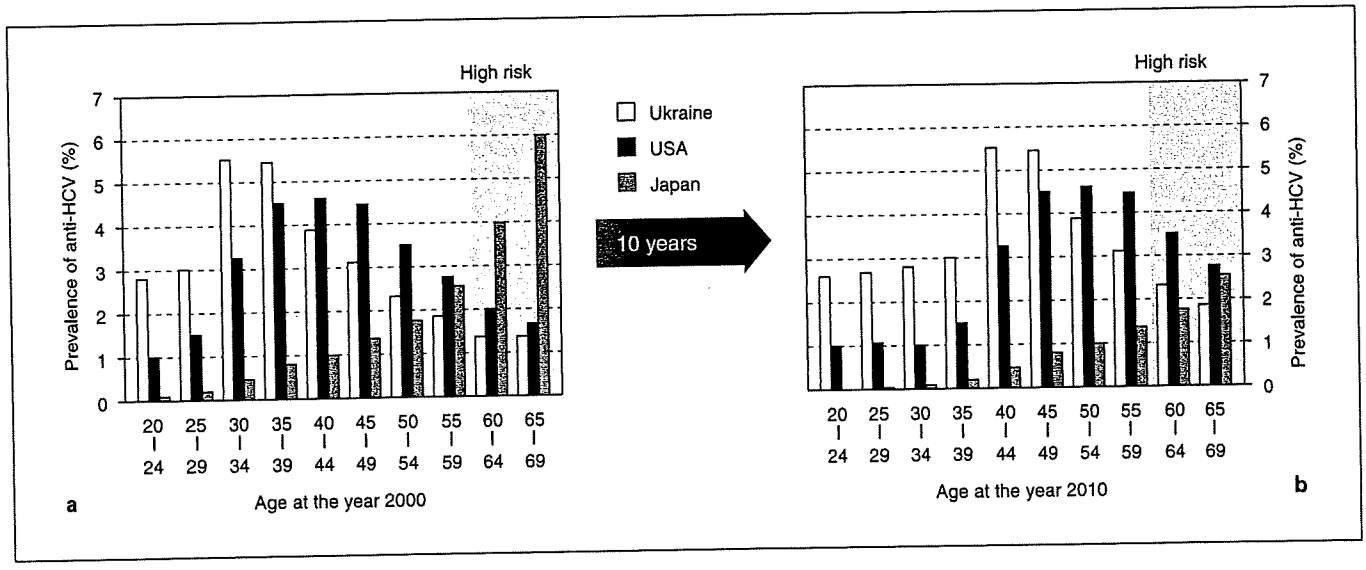


Fig. 10. Age-specific profiles of HCV infection in Japan, the United States and Ukraine. Distributions in the year 2000 (**a**) and those expected in the year 2010 (**b**) are shown.

of anti-HCV steeply increases with age in Japan; the highest is expected in the elderly who scale out of the figure. In contrast, the highest prevalence of anti-HCV is positioned at 40–44 years in the United States, and even younger at 30–35 years in Ukraine.

These wide differences in the age at which anti-HCV peaks among the three countries are attributed to distinct time points when HCV spread extensively among each population. In Japan, HCV infection infiltrated in the subpopulation of younger generations during the 1950s and 1960s in a vicious cycle, but almost completely ceased to occur since 1990 [14]. About two decades later, from the 1970s to the early 1980s, HCV infection widely diffused in the United States; the yearly incidence decreased from 230,000 in the 1980s to 38,000 in the 1990s [22]. More recently, during the late 1980s and the 1990s, HCV infection started to spread in Ukraine and has continued to invade younger generations.

It is of note that the national diffusion of HCV infection is triggered and accelerated by defeats in wars in all the three countries. They are the World War II in Japan, the Vietnam War in the United States and the Afghan War in Ukraine, all of which created a national turmoil and stimulated desperation predominantly in younger generations. What we find in the age-specific distribution of anti-HCV in the three countries at present are long-term sequelae of the wide spread of HCV infection around a major war that are shifting with time.

Age-specific distribution of anti-HCV expected in the year 2010 is depicted by shifting the present profile 10 years toward the future (fig. 10b). In Japan, with essentially no new infections, HCV carriers in their sixties will decrease. On the contrary, many individuals infected with HCV in the United States will go into their sixties along with substantial cases of HCC developing in them. The same will happen in Ukraine after an additional 10 years, which can be projected by the current age-specific prevalence of HCV infection. Should that be the case, these countries are going to follow the profile we have for HCC associated with HCV infection in Japan, with time lags of 10 and 20 years, respectively.

Conclusion

HCC is a rare cancer that is easily preventable. Individuals at high risk for developing HCC are infected with HCV or HBV, of which HCV is more universal, and they are readily identified by viral markers. It is imperative that carriers realize that they are infected with HCV, since the majority are unaware of it. Then, they can consult their present status with hepatologists and receive antiviral treatment for terminating the infection as indicated. HCV carriers who cannot clear infection are regularly followed for liver disease, and those with chronic hepatitis or cirrhosis are screened for HCC at appropriate intervals by noninvasive methods, such as ultrasonography and computed tomography, as well as tumor markers like α-fetoprotein and protein induced by vitamin K absence or antagonist-II. They need to be advised to abstain from alcohol in order to decrease the development of HCC. Antiviral and anti-inflammatory therapies are found effective in decreasing the incidence of HCC, even in patients who fail to clear HCV. Due to an extremely wide variation in the progression of liver disease and the incidence of HCC in HCV carriers, it is imperative to handle the situation on a national basis, as we are conducting in Japan. Efforts will be rewarding, with much less morbidity and mortality caused by HCV infection, and will immensely lessen the economical burden on the nation. Hopefully, interim results of our 5-year national project for spotting HCV carriers in Japan will encourage similar efforts elsewhere in the world where HCV-associated HCC is reasonably expected to increase.

Acknowledgements

We thank the Japanese Red Cross Blood Center for collecting data of the first-time blood donors and their analysis, and the members of the Hiroshima Hepatitis Study Group for follow-up and diagnosis of liver disease in blood donors found with HCV infection at the screening. This study was supported in part by Health and Labour Science Research Grants for Research on Hepatitis and BSE from the Ministry of Health, Labour and Welfare of Japan.

References

1 Lee WM: Hepatitis B virus infection. N Engl J Med 1997;337:1733–1745.
2 Cohen J: The scientific challenge of hepatitis C. Science 1999;285:26–30.
3 Szmuness W, Stevens CE, Harley EJ, Zang EA, Oleszko WR, William DC, Sadovsky R, Morrison JM, Kellner A: Hepatitis B vaccine: Demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. N Engl J Med 1980;303:833–841.
4 Chang MH, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, Liang DC, Shau WY, Chen DS: Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. N Engl J Med 1997;336:1855–1859.
5 Hoofnagle JH: Course and outcome of hepatitis C. Hepatology 2002;36:S21–S29.
6 Hoofnagle JH: Chronic hepatitis B. N Engl J Med 1990;323:337–339.
7 Alter HJ, Seeff LB: Recovery, persistence, and sequelae in hepatitis C virus infection: A perspective on long-term outcome. Semin Liver Dis 2000;20:17–35.
8 Seeff LB: Natural history of chronic hepatitis C. Hepatology 2002;36:S35–S46.
9 Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH, Alter HJ: Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: Analysis by detection of antibody to hepatitis C virus. Hepatology 1990;12:671–675.
10 Tong MJ, el-Farra NS, Reikes AR, Co RL: Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 1995;332:1463–1466.
11 Kenny-Walsh E: Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. N Engl J Med 1999;340:1228–1233.
12 Vogt M, Lang T, Frosner G, Klingler C, Sendl AF, Zeller A, Wiebecke B, Langer B, Meisner H, Hess J: Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med 1999;341:866–870.
13 Hamada H, Yatsuhashi H, Yano K, Daikoku M, Arisawa K, Inoue O, Koga M, Nakata K, Eguchi K, Yano M: Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. Cancer 2002;95:331–339.
14 Yoshizawa H: Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: Projection to other countries in the foreseeable future. Oncology 2002;62:S8–S17.
15 Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, Suzuki K, Miyakawa Y, Yoshizawa H: Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995–2000. Intervirology 2004;47:32–40.
16 Iida F, Iida R, Kamijo H, Takaso K, Miyazaki Y, Funabashi W, Tsuchiya K, Matsumoto Y: Chronic Japanese schistosomiasis and hepatocellular carcinoma: Ten years of follow-up in Yamanashi Prefecture, Japan. Bull World Health Organ 1999;77:573–581.
17 Tanaka Y, Hanada K, Orito E, Akahane Y, Chayama K, Yoshizawa H, Sata M, Ohta N, Miyakawa Y, Gojobori T, Mizokami M: Molecular evolutionary analyses implicate injection treatment for schistosomiasis in the initial hepatitis C endemics of Japan. J Hepatol 2005;42:47–53.
18 Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Aly Ohn ES, Anwar W, Sallam I: The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000;355:887–891.
19 Yoshizawa H, Tanaka J: A national project for the management of viral hepatitis toward prevention of hepatocellular carcinoma in Japan; in Morrissey RF (ed): International Kilmer Conference Proceedings. Laval, Polyscience Publications, 2004, vol 8, pp 247–264.
20 Beck JR: Markov models of natural history. J Clin Epidemiol 1988;41:619–621.
21 Tanaka J, Kumada H, Ikeda K, Chayama K, Mizui M, Hino K, Katayama K, Kumagai J, Komiya Y, Miyakawa Y, Yoshizawa H: Natural histories of hepatitis C virus infection in men and women simulated by the Markov model. J Med Virol 2003;70:378–386.
22 Williams I: Epidemiology of hepatitis C in the United States. Am J Med 1999;107:S2–S9.

ELSEVIER

Journal of Clinical Virology 36 Suppl. 1 (2006) S56–S64

JOURNAL OF CLINICAL VIROLOGY

www.elsevier.com/locate/jcv

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

Hisao Yugi[a], Masaaki Mizui[b], Junko Tanaka[c], Hiroshi Yoshizawa[c,*]

[a] *Department of NAT, Central Blood Institute, Japanese Red Cross Society, Tokyo, Japan*
[b] *Japanese Red Cross Hiroshima Blood Center, Hiroshima, Japan*
[c] *Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan*

Keywords: HBsAg; anti-HBc antibody; HBV DNA; NAT; Blood screening

1. Introduction

The hepatitis B virus (HBV) screening strategy can be adapted to the regional epidemiological situation and can differ between countries with a high or a low prevalence of HBV infection. The prevalence of HBV infection is known to be higher in Asian countries, including Japan, than in Northern Europe and North America (Tanaka et al., 2004; see also the paper by Liu et al. in this supplement).

The regional HBV epidemiology is determined by the following three parameters:

(1) The prevalence of hepatitis B surface antigen (HBsAg): HBV or HBsAg carrier rate.
(2) The prevalence of anti-hepatitis B core (anti-HBc) antibody with or without anti-hepatitis B surface (anti-HBs) antibody: Rate of anamnestic HBV infection.
(3) The incidence of HBV infection: Rate of new HBV infections.

This paper presents the current HBV blood screening strategy in Japan as could be established on the basis of the three parameters listed above and explains the rationale of the screening strategy according to new insights in the natural course of acute and chronic HBV infection.

2. Strategy for HBV blood screening in HBV carriers

Most individuals who are found to be positive for HBsAg after blood donation or through a health check-up are considered to be chronic HBV carriers, and only few are new acute infections that may resolve within the next months. In general, the concentration of HBsAg, as well as the titer of anti-HBc antibody, is high in the blood of chronic HBV carriers.

Usually, HBV carriers produce anti-HBs antibody, but it is used up by the large amount of HBsAg being secreted from the HBV-infected liver, and therefore anti-HBs is often not detectable in serological testing (Fig. 1).

Consequently, to achieve the aim of blood safety, screening for HBsAg is sufficient for identifying these typical HBV carriers, and HBV DNA screening by nucleic acid amplification testing (NAT) has no added value. It is also not very effective to introduce a more sensitive reagent to detect trace amounts of HBsAg, because the level of HBsAg in HBV carriers is usually very high. On the other hand there is a group of so-called tail-end HBV carriers that over many years enter into a low replicative stage and have lost detectable HBsAg. In these isolated anti-HBc reactive individuals HBV-NAT is the only direct marker for potential infectivity of the blood.

In November 1989, well before HBV-NAT blood screening was technically feasible, the Japanese Red Cross (JRC) Blood Center introduced a screening method that eliminates blood with an anti-HBc antibody haemagglutination inhibition (HI) titer of 2^6 or more, despite a negative result for HBsAg, and this screening algorithm successfully decreased the incidence of transfusion-transmitted HBV infection. (JRC non-A, non-B Hepatitis Research Group, 1991).

The introduction of this screening protocol was based upon the report that HBV DNA is often detected in blood with an anti-HBc antibody titer of at least 2^6 HI or more, even when HBsAg is not detected (Iizuka et al., 1992). So, it is expected that the Japanese anti-HBc screening system has screened out the HBV DNA reactive donors with isolated high anti-HBc titres, who most probably represent the group of tail-end HBV carriers who over time have entered a low replicative state and no longer produce detectable HBsAg.

* Corresponding author. Hiroshi Yoshizawa, Department Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, 734-8551, Japan. Tel: +81 82 257 5162; fax: +81 82 257 5164.
E-mail address: eidcp@hiroshima-u.ac.jp (H. Yoshizawa).

1590-8658/ $ – see front matter

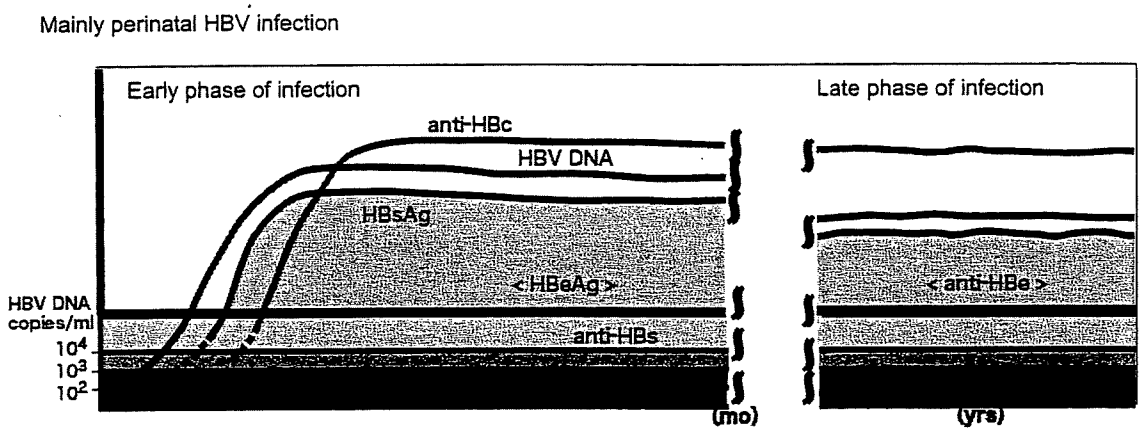


Fig. 1. Course of HBV markers in classical persistent HBV infection as often develops in infants born to HBeAg positive HBV carrier mothers in Asia. Over the years HBV carriers can seroconvert to anti-HBe and enter a lower replicative state (HBV DNA load $<10^6$ cps/ml) in the peripheral blood. Some HBV carriers at older age enter into a very low replicative state (HBV DNA $<10^3$ cps/ml) and no longer produce detectable HBsAg (not illustrated in figure).

3. HBV DNA testing by NAT, and characteristics of HBV-DNA positive donors

The JRC annually conducts NAT blood screening on more than 5.7 million samples of donated blood. From the introduction of this system in July 1999 until January 31, 2000, 500 samples of donated blood were pooled as a single batch to be tested by NAT (500 mini-pool NAT). In February 2000 the batch size was reduced to 50 donations (50 mini-pool NAT), and then to 20 (20 mini-pool NAT) in August 2004, which is still the present minipool size.

Of the total of 33 735 075 seronegative blood donations tested by NAT between July 1999 and December 31, 2005, 625 samples were HBV DNA-positive, 93 were HCV RNA-positive and 12 were HIV RNA-positive.

To interpret these results correctly for HBV it is important to understand the HBV screening algorithm of the JRC in more detail (Fig. 2). In the JRC, only the blood of HBsAg negative and anti-HBc antibody negative donors or donors who have an anti-HBc HI antibody titer of 2^4 or less is subjected to final safety testing by HBV-NAT.

In addition, blood donations with anti-HBc HI antibody titers of 2^5 or more are eligible for HBV-NAT screening if the level of anti-HBs antibody is found to be 200 mIU/ml or more (Fig. 2). The sensitivity of the HBV DNA tests using the NAT system currently employed by the JRC is approximately 10^2 copies/ml (95% hit rate cut-off point, Sato et al., 2001).

Thus, in a routine test by the 20 mini-pool NAT system, a sample is considered to be HBV DNA-positive if blood with a HBV DNA level of approximately 10^3 copies/ml or more is present in the mixture. In this case, NAT tests for HBV DNA detection are conducted on the plasma of each of the

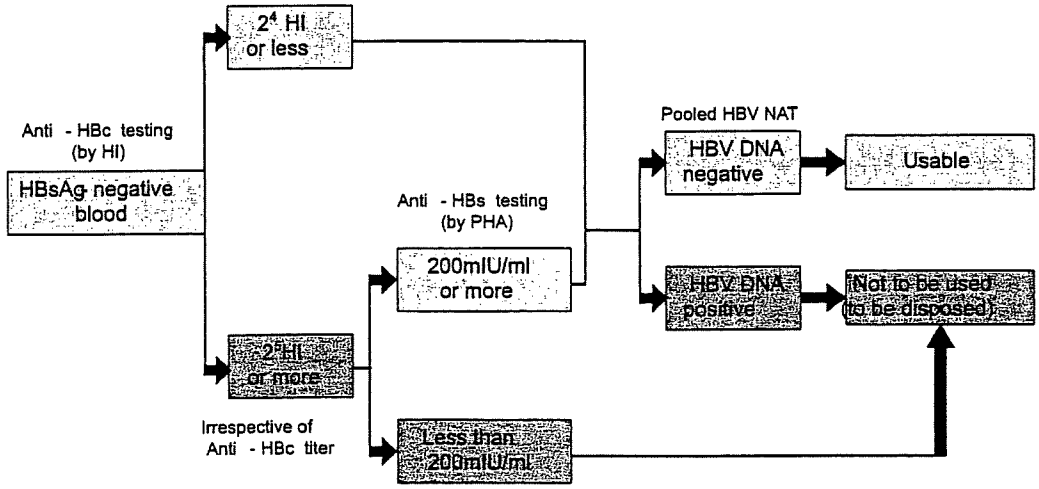


Fig. 2. Viral serology and NAT screening algorithm used by the Japanese Red Cross (JRC). Only donations that are considered serology negative for HBsAg, anti-HBc, anti-HCV and anti-HIV are subjected to minipool NAT screening. Since 1989 JRC tests donations also for anti-HBc and anti-HBs titre. Anti-HBc reactive donations with haemagglutionation inhibition (HI) titres of 2^5 or higher are discarded, unless the anti-HBs titre in PHA is higher than 200 mIU/ml. Only anti-HBc reactive donations with HI titres $<2^5$ or donations with anti-HBc HI titres $>2^4$ and anti-HBs titres >200 mIU/ml are subjected to minipool NAT screening.

20 donors (individual-donation NAT), and the HBV DNA-positive donor is identified. With the procedure described above, a total of 625 of 33 735 075 blood donations were determined to be HBV DNA-positive between July 1999 and December 2005 (rate 1:54 000). This rate becomes 2–3-fold lower when more sensitive HBsAg screening (Abbott, PRISM) is performed on these samples (Minegishi et al., 2003). Of these 625 donors (minus one sample that could not be analyzed owing to breakage of the blood bag), we have tabulated the numbers of HBV DNA-positive donors and the prevalence of the anti-HBc antibody according to age group. Table 1 shows that HBV DNA-positive donors were mostly (490/624: 78.5%) under 40 years of age, with only 134 of 624 (21.5%) above age 40.

Of the younger donors (up to 39 years old), all but five tested negative for the anti-HBc antibody (485/490: 99.0%). Probably these HBV DNA yield cases represent pre-HBsAg window phase infections that were interdicted by the JRC screening system.

Donors of 40 years of age and above, by contrast, showed a high prevalence of anti-HBc antibody (59/134:44.0%) – especially those of 50 years of age and above, of whom over 64.0% (48/75) tested positive. It must be emphasized that these anti-HBc positive donors all had very low anti-HBc HI titres of 2^4 or less. The majority of silent HBV carriers with higher anti-HBc titres had probably already been screened out of the blood supply since 1989 (Iizuka et al., 1992).

Originally, HBV DNA testing with NAT was introduced in order to further improve blood safety by early detection of acute HBV infection before the onset of HBsAg. Indeed, the JRC minipool NAT test strategy has identified 560 anti-HBc negative donations (rate 1:60,000) that are likely to be drawn in the pre-HBsAg window phase. However, our results show that HBV DNA testing by NAT could detect acute HBV infection not only in its early phase – before the emergence of HBsAg – mainly in the younger age groups, but also in its late phase – after the disappearance of HBsAg – mainly in the middle and older age groups.

Thus, it may be necessary, in order to further improve the safety of donated blood using HBV DNA testing with NAT, to develop a strategy based on good understanding of the natural courses of both early and late phases of acute and chronic HBV infection. There are two different categories of HBsAg negative, but anti-HBc reactive and (low) viremic donors (Allain, 2004):

(1) The first group are the tail-end HBsAg carriers who after many years in the HBeAg negative phase enter into a low replicative stage and no longer produce detectable HBsAg.
(2) The second group may have serologically cleared HBV infection in the past, but after anti-HBs titres drop over the years, low-level HBV replication in the liver may become detectable again in plasma with sensitive NAT.

Probably the vast majority of the the first category of silent HBV carriers have been screened out of the Japanese blood supply since 1989 by the anti-HBc screening algorithm and these donors have never been subjected to the HBV DNA screening algorithm (see Fig. 2). The group of recently identified HBV DNA positive donors with low anti-HBc titres more likely belong to the second category of late acute phase infections, although it cannot be excluded that some of them are in the first category of tail-end carriers with anti-HBc HI titres that dropped below 2^4 or less.

Table 1
Age-specific rates of late-phase HBV infection (anti HBc-positive) in the 624 HBV DNA-positive donors found by NAT in Japan[a]

Age group	HBV DNA positives by NAT		Anti-HBc positive (%)[b]		
10–19	67	490/624 (75.8%)	2	(3.0)	5/490 (1.0%)
20–29	285		3	(1.1)	
30–39	138		0		
40–49	59	134/624 (21.5%)	11	(18.6)	59/134 (44.0%)
50–59	50		26	(52.0)	
60–69	25		22	(88.0)	
Total	624		64	(10.3)	

[a] JRC 1997.7–2005.12 – No. of blood samples tested: 33 735 075.
[b] Anti-HBc positives: by AxSYM®; Anti-HBc titer: 2^4 HI or less.

4. Natural course of acute HBV infection

When an adult contracts an acute HBV infection, after some incubation time, HBV DNA emerges first in the peripheral bloodstream, and HBsAg follows. HBsAg can be detected in the peripheral bloodstream for a few months, but gradually disappears thereafter. Following this, the HBV DNA also eventually dissipates, the HBV infection subsides, and the host regains health and acquires immunity (see Fig. 3).

In this way, it was considered that, after an episode of acute HBV infection, the patient would be clinically in complete recovery. Serologically, such an infection was interpreted as anamnestic, and because of the immunity acquired, there would be no future possibility of re-infection of that patient. However, in recent years reports of HBV infections in recipients of liver transplants from donors in whom anti-HBc antibody has been found, despite the failure to detect HBsAg or HBV DNA in the peripheral bloodstream, have led to further research using molecular-biology techniques (Dodson et al., 1997; Uemoto et al., 1998). As a result, it is now clear that, after the termination of an acute (or transient) HBV infection, a lifelong presence of minute amounts of HBV infection remains in the cytoplasm of the hepatocytes (Marusawa et al., 2000). In other words, it is necessary to add a molecular virological aspect to our current clinical, virological and serological

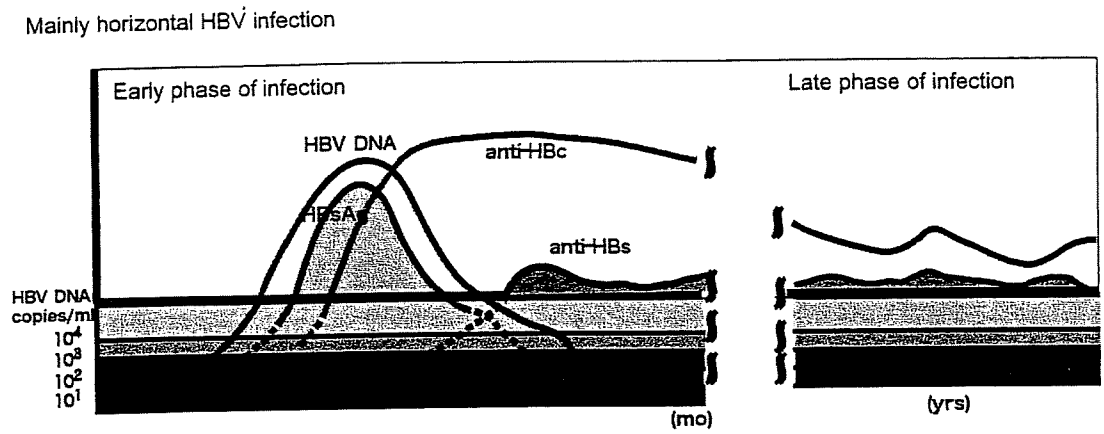


Fig. 3. Natural course of HBV markers in acute resolved HBV infection, often observed in Asia after horizontal transmission in children and adults. Patients mount an anamnestic immune response and with appearance of neutralizing anti-HBs in serum it was believed that HBV was cleared from the liver and the blood was no longer infectious. More recently with application of molecular techniques it has become clear that HBV persists in the liver (latent infection) and that after years, when the neutralizing anti-HBs titres have dropped (usually <20 mIU/ml), low-level HBV replication can occur and may be detected again by sensitive HBV DNA NAT assays, and as a result the blood could become infectious again.

knowledge of the natural course of acute HBV infections, so as to come to a new understanding based on a new concept (Fig. 3).

4.1. Virological and serological characteristics of the early phase of acute HBV infections

To ascertain the limits of effectiveness of HBV DNA testing by 20-mini-pool NAT, a detailed understanding of the natural course of the early phase of an acute HBV infection is needed.

For this purpose, an HBV (genotype A) infection experiment was conducted using chimpanzees that have been involved in HCV infection studies (Katayama et al., 2004; Tanaka et al., 2005), and the following results were obtained (unpublished data):

(1) When the blood sample in the early phase of an acute HBV infection (before the emergence of anti-HBc antibody response) was used as an inoculum, the minimum infective dose of HBV (MIDH) was equivalent to 10 copies of HBV DNA determined by NAT in vitro.
(2) The NAT window period (the time taken for the HBV DNA titer in the peripheral blood to reach 10^2 copies/ml as the individual NAT detection sensitivity) in chimpanzees in which infection was established by inoculation of the MIDH, was 55–70 days.
(3) In chimpanzees in the early phase of acute HBV infections, the log time required for the HBV DNA titer in the peripheral blood to increase tenfold was 7.7–8.6 days, the maximum possible time being 10 days.

The sensitivity of HBV DNA in 20 mini-pool NAT is 10^3 copies/ml when calculated per serum sample. Thus, the window period of 20 mini-pool NAT is calculated to be 65–80 days.

However, the window period of HBsAg in a chimpanzee subjected to the MIDH is 80–100 days, even if the most sensitive HBsAg detecting reagent (PRISM®, Abbott, USA) is used.

Figure 4 presents the details of the natural course of the early phase of acute HBV infection based on the evidence acquired in virological, serological, as well as molecular-virological studies.

Thus, by the introduction of HBV DNA testing using the 20 mini-pool NAT screening for HBV the infectious window period has been shortened by at least 20 days, in comparison with HBsAg screening in donors having early-phase acute HBV infections.

On the other hand, if donors who have been exposed to the MIDH would donate blood shortly after such an exposure, the present HBV DNA testing procedure (involving the 20 mini-pool NAT), could not screen out blood carrying a risk for up to 80 days.

In addition, even if the HBV DNA testing by 20 mini-pool NAT were changed to HBV DNA testing by individual NATs with the sensitivity increased tenfold, the window period would be shortened by only 10 days at most, and the tests would still not detect the risk for up to 70 days.

It must be borne in mind however that a considerable amount of time may elapse during the early stage of viral replication in the liver in which the blood does not contain enough virus to be infectious. According to our unpublished chimpanzee studies it is expected that the infectious window period starts when a blood component contains approximately 10 virions or HBV-DNA copies. If a red cell concentrate contains ~10 ml of plasma the infectious window period would thus start at a concentration of 1 HBV-DNA copy/ml. So it would take two log times or 20 days to reach the time point that individual-donation NAT would be able to detect infection 95% of the time. Elsewhere in this supplement Busch and Kleinman describe this new approach of window period risk modeling based on the viral growth curve during early onset of viremia

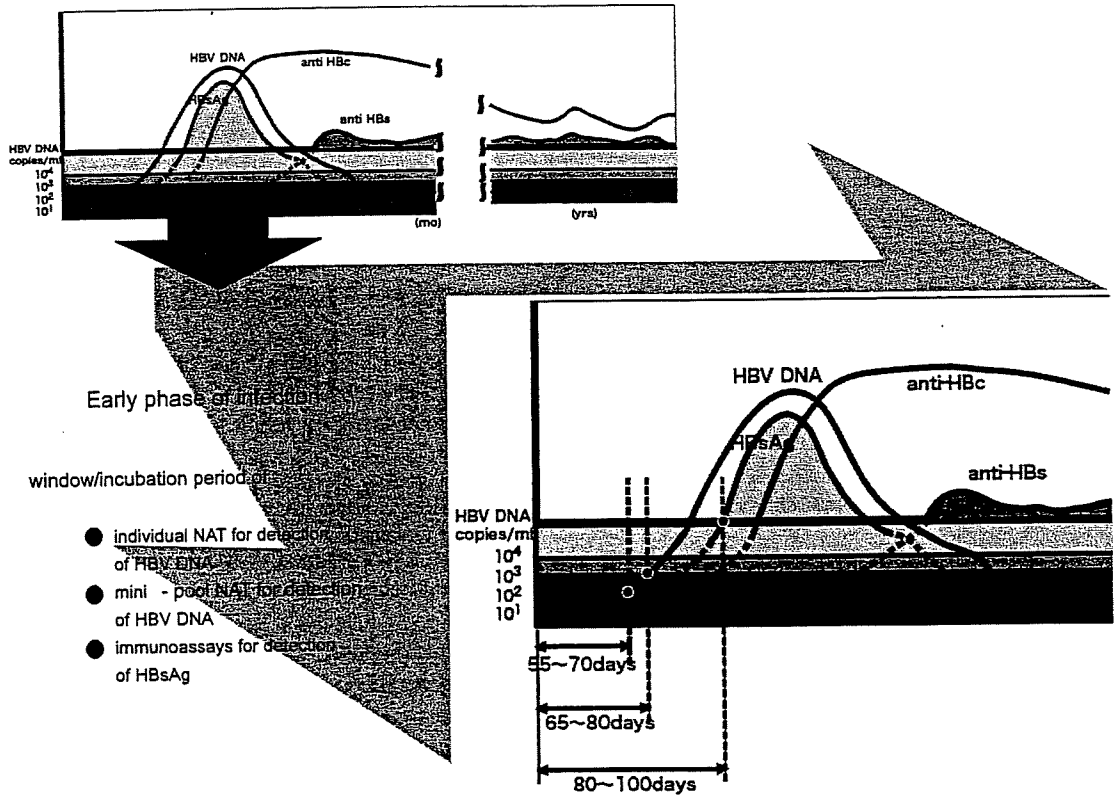


Fig. 4. Conceptual illustration of events during early acute HBV infection and estimated sensitivity of JRC HBV-NAT screening in the pre-HBsAg window period as modeled on the basis of our unpublished chimpanzee HBV (genotype A) infection experiments. The estimates of the window periods of 80–100 days before HBsAg seroconversion (PRISM), 65–80 days to the minipool NAT seroconversion point (10^3 cps/ml) and 55–70 days to individual donation NAT conversion (cut off ~10^2 cps/ml) were based on the viral growth curves seen in chimpanzees infected with the minimum infectious dose of approximately 10 copies HBV (MIDH). In recent risk models it is assumed that blood drawn in the early window period may not be infectious as long as the viral concentration has not increased to a level that equals the MIDH of ~10 HBV DNA copies transfused by the blood components. For example, the infectious window period may start when the virus concentration reaches a level of 1 copy/ml HBV DNA for red-cell transfusions with ~10 ml of plasma per packed cell unit.

and thereby assume that the NAT seroconversion point is at a concentration that is detectable 50% of the time. For more accurate mathematical modeling one has to take into account (1) the probability curve of detecting low copy numbers of virus by NAT and (2) the chance that these minute amounts of undetectable viral nucleic acid are representing enough replication-fit virus in a transfused blood component. (Weusten et al., 2002). Indeed, look-back studies by Satake et al. (2005) in seroconverting donors in the JRC screening algorithm showed that 50% of individual-donation NAT positive and anti-HBc negative window period donations were non-infectious despite infusion of much higher doses than the MIDH of ~10 copies of HBV DNA that was established in our chimpanzee studies. Although the recipients in the JRC look-back study were considered to be susceptible to HBV infection on the basis of serological results in pre-transfusion samples, it cannot be excluded that some of them had in fact acquired low-level immunity. Another explanation for the fact that 50% of the presumed window period donations was not infectious in the recipients is that the infectivity of HBV was reduced during storage of blood components or that the infectivity of HBV was neutralized by anti-HBs derived from blood components being administered simultaneously.

4.2. *Virological and serological characteristics of late-phase acute and chronic HBV infections*

The HBV infection in which HBV DNA can be detected but HBsAg cannot, has been referred to as "occult HBV infection" or "an atypical HBV carrier state", and by many other expressions. In this paper, where HBV infected donors are found to carry both HBV DNA and the anti-HBc antibody, but are HBsAg negative, they are referred to simply as late-phase acute or chronic HBV infections. Indeed the NAT yield cases of the JRC most likely are late-phase acute resolved infections, although it cannot be excluded that some of them are tail-end carriers with subdetectable HBsAg production who never have seroconverted to anti-HBs.

In the late-phase acute and chronic HBV infection groups, the positive rate of HBV DNA by NAT in the peripheral blood (the frequency with which the concentration of HBV DNA exceeds 10^2 copies/ml) depends upon the anti-HBc antibody titer (Iizuka et al., 1992). In a recent

Table 2
HBV DNA-positive rates in donated blood positive for anti-HBc, but negative for HBsAg and anti-HBs[a]

Anti-HBc titer 2^N by HI[b]	Number tested	HBV-DNA positives[c]	
		n	(%)
12 †	5	5	(100.0)
11	20	17	(85.0)
10	31	19	(61.3)
9	48	24	(50.0)
8	54	27	(50.0)
7	72	17	(23.6)
6	96	23	(24.0)
5	117	9	(7.7)
4	20	2	(10.0)
Total	463	143	(30.8)

[a] Blood positive only for anti-HBc was collected selectively.
[b] Hemagglutination inhibition (in-house reagent of JRC).
[c] 10^2 copies or more.

study among isolated anti-HBc positive donors (Table 2) we were able to confirm that plasma viremia is more frequently observed in groups with a high anti-HBc antibody titer. In the group of donors with high anti-HBc titres who were anti-HBs negative and who are normally not subjected to the JRC HBV-NAT screening system the proportion of HBV-NAT positives was very high and ranged from 25% to 100% with increasing anti-HBc HI titres from 2^6 to 2^{12}. So, Table 2 reminds us again that the vast majority of HBV DNA reactive and potentially infectious anti-HBc reactive donors is already screened out of the blood supply by the JRC anti-HBc HI screening algorithm. As stated before, the natural history of these viremic individuals with high anti-HBc titres may be totally different from that of the individuals with low anti-HBc titres more recently picked up by the JRC NAT screening system. It may be that the anti-HBc low titre HBV-NAT yield cases of JRC represent the group of acute resolved infections being in a late stage with recurrent low-level viremia.

In addition, instead of individual NAT, when HBV DNA testing by NAT is attempted after concentration of HBV from a large volume of sample, HBV DNA can be detected more often in groups with low anti-HBc antibody titers. When HBV DNA was extracted from 2 ml of plasma of 500 donors with isolated low titre anti-HBc reactivity (2^4 or less by HI) 8 of 500 were positive for HBV DNA, 4 of whom had HBV DNA levels of 10 copies/ml or less.

This suggests that, in order to secure maximum safety from the late stage of an acute HBV infection, the ideal strategy would be to test for anti-HBc antibody by highly sensitive serological tests, and to eliminate all blood that tests positive for anti-HBc antibody, rather than relying on HBV DNA testing by NAT.

This would be a rational system in areas with a low HBV exposure rate (low anti-HBc antibody positive rate), such as North America and Europe. However, in Asian countries such as Japan, it would not be realistic to try to eliminate all blood that tests positive for anti-HBc antibody, as many healthy and safe donors with both anti-HBc and anti-HBs antibodies would be lost for the blood supply. Table 3 shows how many donors with anti-HBc (and anti-HBs) antibodies can still be found among the donor population in the JRC Hiroshima Blood Center, as measured by a sensitive reagent (AxSYM®, Abbott, USA).

Overall, a high anti-HBc detection rate of 19.5% is found, and in donors of 50 years of age and older, the anti-HBc antibody positive rate is very high – 30% or more.

In contrast to the estimated anti-HBc prevalence of ~20% in Japanese blood donors, between July 1999 and December 2005 only few samples tested positive for both anti-HBc antibody and for HBV DNA in the JRC NAT screening system (donated blood showing an HBV DNA level of 10^3 copies/ml or more, as determined by mini-pool NAT). The minipool NAT detection rate in low-titre anti-HBc donors not interdicted by serology testing was

Table 3
Age-specific anti-HBc positive rate in HbsAg negative blood donors (anamnestic infections) in Hiroshima[a]

Age group	Total			Male			Female		
	No. of donors	Anti-HBc positives (%)[b]		No. of donors	Anti-HBc positives (%)[b]		No. of donors	Anti-HBc positives (%)[b]	
⩽19	40	3	(7.5)	20	3	(15.0)	20	0	
20–29	40	2	(5.0)	20	1	(5.0)	20	1	(5.0)
30–39	40	3	(7.8)	20	2	(10.0)	20	1	(5.0)
40–49	98	12	(12.2)	50	7	(14.0)	48	5	(10.4)
50–59	96	32	(33.3)	60	26	(43.3)	36	6	(16.7)
⩾60	61	21	(34.4)	31	9	(29.0)	30	12	(40.0)
Total	375	73	(19.5)	201	48	(23.9)	174	25	(14.4)

[a] Hiroshima Red Cross Blood Center, 2001 – 1 data per 1 blood donor.
[b] Anti-Hbc positives: by AxSYM®.

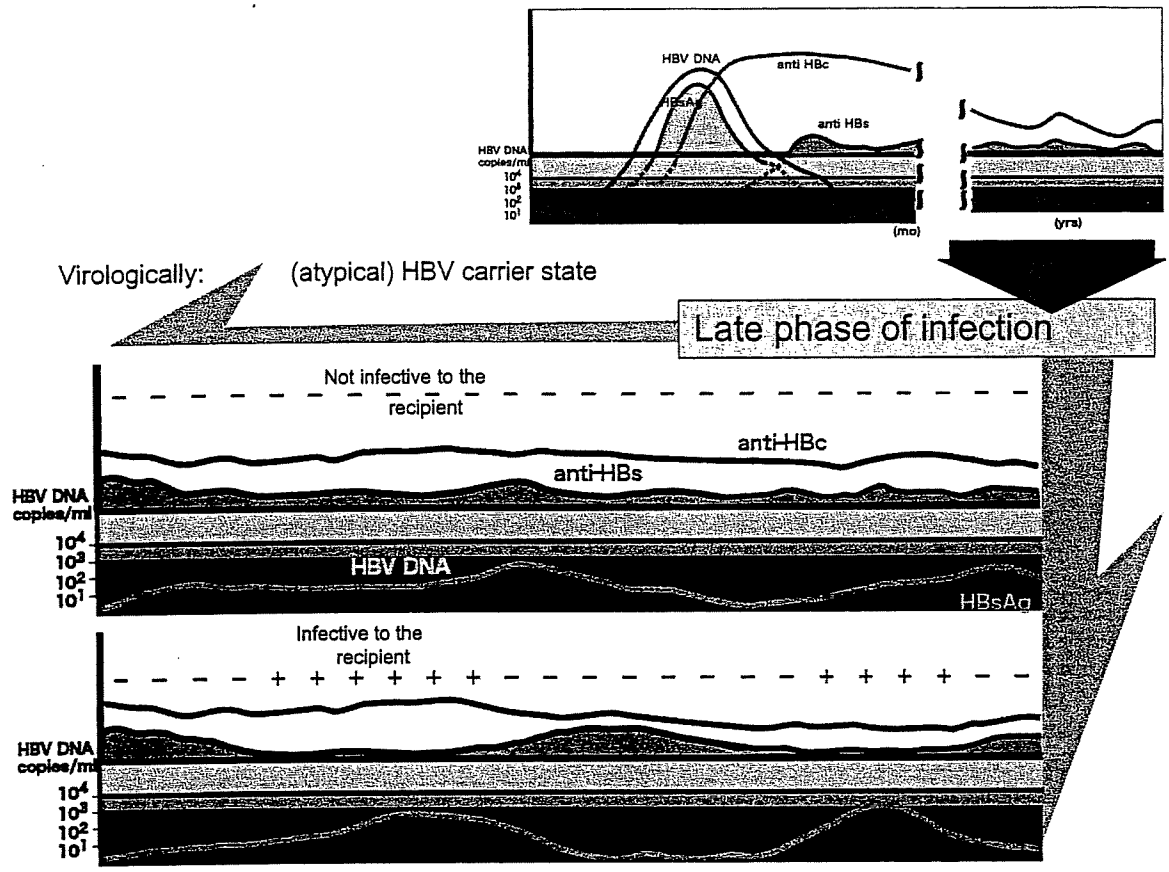


Fig. 5. Conceptual illustration of the course of fluctuating HBV markers in a late phase after acute resolved HBV infection. Although protective anti-HBs antibodies have been developed after clearance of HBV the virus persists latently in the liver. When after many years the immune response fades and neutralizing circulating anti-HBs antibodies drop to subdetectable levels (<20 mIU/ml) the infection may re-activate and low-level viremia in plasma may become detectable by sensitive HBV-NAT. The re-activation of HBV and HBsAg production may evoke a new secondary immune response and the viral load may drop again. The infectivity of the blood of these individuals in the HBV DNA positive stage of recurrent HBV replication in the liver depends on the level of immune neutralization by antibodies complexed to the HBV virions.

only 64 in 33 735 075 donations screened at the JRC (rate 1:527 000).

A retrospective study carried out by the JRC (Satake et al., 2005) has shown that since the present form of HBV DNA testing using NAT has been introduced, transfusion-transmitted HBV infections caused by blood from subjects with late-phase acute or chronic HBV infections have been quite rare. Only one anti-HBc positive low-viremic case has been reported that was infectious, whereas the vast majority (97%) of individual-donation NAT reactive look-back cases was not (Satake et al., 2005). Interestingly, Inaba et al. (2005) reported multiple HBV transmissions caused by subsequent donations of a platelet apheresis donor who was anti-HBc low-reactive and who retrospectively tested HBV-NAT negative ($<10^2$ cps/ml).

These results indicate that, in countries such as Japan, where there is a high prevalence of the anti-HBc antibody in donor blood, for optimal safety it is advisable to implement a screening strategy combining anti-HBc antibody measurement with HBV DNA testing using NAT.

Figure 5 demonstrates the virological and serological characteristics that occur during the late phase of an acute HBV infection. In the majority of cases, low levels of anti-HBc antibodies and anti-HBs antibodies coexist, but the subject has fully recovered clinically, and serologically shows an anamnestic infection.This is how the situation has been understood hitherto; and there is no need to alter our understanding of this scenario.

Most recipients of such blood will not contract HBV from transfusions. However, if accelerated proliferation of the HBV occurs in the hepatocytes, a certain amount of HBsAg would be secreted into the bloodstream together with HBV and then, anti-HBs antibodies would be consumed because of the formation of immune complexes with HBsAg, as well as with HBV, and thus fall in titer, and the risk of infection for the recipient will increase. In fact, a similar pattern of fluctuating HBV markers may persist in tail-end HBV carriers that have never mounted an anamnestic response after HBV exposure. It is expected, however, that in this category of chronic tail-end carriers the weak anti-HBs response may not completely neutralize the virus, which makes the blood more infectious.

In general, it is speculated that blood carrying a late-phase acute HBV infection has a lower infectivity than

uch blood drawn in an early-phase-acute HBV infection, ɔecause of the formation of immune complexes between ⊣BV and neutralizing anti-HBs antibodies. Prince et al. 2001) demonstrated that HBV DNA positive blood with ɔnly detectable anti-HBc and anti-HBs was not infectious n chimpanzees. However, the MIDH for blood with late-ɔhase acute or chronic HBV infections with isolated anti-⊣Bc antibody without detectable anti-HBs has not yet been ɛstablished in chimpanzees, and may vary from case to ɔase. Therefore, look-back studies at JRC (Satake et al., manuscript in preparation) are important for understanding the residual risk of HBV transmission in the current Japanese screening setting.

5. Feasibility of further improvement of blood safety

In this paper we have summarized the Japanese screening strategy for HBV carriers, early-phase acute HBV infections, and late-phase acute or chronic HBV infections, and speculate on future alternative ways to further improve and guarantee the HBV blood safety in Japan.

Blood screening for the typical HBV carrier requires the HBsAg test alone, and HBV DNA testing by NAT is unnecessary. However there is a group of HBV carriers that after decades enters into a low-replicative phase in which no longer detectable HBsAg is produced. For these cases HBV-NAT testing is useful. Probably most of these cases have relatively high anti-HBc titres and low anti-HBs titres and therefore have already been screened out of the Japanese blood supply by the JRC serological screening algorithm introduced in 1989.

Blood in the early phase of an acute HBV infection is highly infectious, and the MIDH is equivalent to 10 copies of HBV DNA determined by NAT in vitro. This level of HBV cannot be reliably detected even by HBV DNA testing using individual NAT.

In an experiment using chimpanzees, it was seen that it would take a minimum of 70 days for the HBV DNA level in the peripheral blood to reach the detection level of 10^2 copies/ml, with the HBV DNA testing using individual NAT, and a minimum of 80 days by means of the present HBV DNA testing by 20 mini-pool NAT. However, it has to be borne in mind that during a relatively large part of the pre-HBV DNA seroconversion window period the blood probably contains sub-infectious levels of HBV DNA (<1 copy/ml).

To achieve further improvement in blood safety, it is necessary to educate blood donors about the danger of donating blood soon (less than 3 months) after high-risk behavior for HBV infection, in order to eliminate blood donations with early-phase acute HBV infections. In addition, it is important to measure the incidence rate of HBV infections in donor groups and to determine the major risk factors that cause an HBV infection in each country. These sero-epidemiological results should then be used for the education of blood donors.

For the dismissal of blood with late-phase acute HBV infections in countries with a low prevalence of the anti-HBc antibody, it is sufficient to eliminate all blood testing positive for the anti-HBc antibody, using sensitive detection methods. In contrast, in countries with a high prevalence of the anti-HBc antibody, it is advisable that a combined blood screening approach involving HBV DNA testing using NAT and anti-HBc antibody measurement be implemented.

It is suspected that the HBV in blood with a late-phase acute HBV infection will form immune complexes with neutralizing anti-HBs antibodies, making the the blood less infectious. Probably a logarithmically higher HBV level is required for infection of immune-complexed HBV than in the case of free HBV in early-phase infections. Consequently, it is speculated that by increasing the HBV DNA detection sensitivity by tenfold, most (if not all) transfusion-transmitted HBV infections in blood with late-phase of acute HBV infections can be eliminated.

Acknowledgement

This work was done together with the members of Japanese Red Cross NAT study group, the study group for NAT standardization, as well as the study group for epidemiology on HBV and HCV infection, under the auspices of the Ministry of Health, Labor and Welfare of Japan.

We appreciate very much the efforts of Dr.Andrew Heaton and Dr. Nico Lelie to present our experience in blood safety testing in Japan in this JCV supplement and thank Dr. Nico Lelie for giving us kind advice and providing assistance to prepare this manuscript.

Part of this work has been presented at an ISBT satellite symposium in Athens, Greece in 2005.

References

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

Dodson SF, Issa S, Araya V, Gayowski T, Pinna A, Eghtesad B, et al. Infectivity of hepatic allografts with antibodies to hepatitis B virus. Transplantation 1997;64:1582–4.

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

Inaba S, MiyataY, Ishii H, Kajimoto S, Yugi H, Hino M, et al. Hepatitis B transmission from an occult HB virus carrier, Transfusion 2005; 45(Suppl);94A.

JRC Non A, non B Hepatitis Research Group. Effect of screening for hepatitis B virus antibody and hepatitis B virus core antibody on incidence of post transfusion hepatitis. Lancet 1991;338:1040–1.

Katayama K, Kumagai J, Komiya Y, Mizui M, Yugi H, Kishimoto S, et al. Titration of hepatitis C virus in chimpanzees for determining the copy number required for transmission. Intervirology 2004;47:57–64.

Marusawa H, Uemoto S, Hijikata M, Ueda Y, Tanaka K, Shimotohno K, et al. Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen. Hepatology 2000;31:488–95.

Minegishi K, Yoshikawa A, Kishimoto S, Yugi H, Yokoya N, Sakurada M, et al. Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay for hepatitis B surface antigen screening. Vox Sang 2003;84:287–91.

Prince AM, Lee DH, Brotman B. Infectivity of blood from PCR-positive, HBsAg negative, anti-HBs positive cases of resolved hepatitis B infection. Transfusion 2001;41:329–32.

Satake M, Taira R, Yugi H, Tadokoro K. Lookback study for transfusion-related HBV infection in Japan. Transfusion 2005;45(Suppl):9A (abstract).

Sato S, Ohhashi W, Ihara H, Sakaya S, Kato T, Ikeda H. Comparison of the sensitivity of NAT using pooled donor samples for HBV and that of serological HBsAg assay. Transfusion 2001;41;1107–13.

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

Tanaka J, Katayama K, Kumagai J, Komiya Y, Yugi H, Kishimoto S, et al. Early dynamics of hepatitis C virus in the circulation of chimpanzees with experimental infection. Intervirology 2005;48:120–3.

Uemoto S, Sugiyama K, Marusawa H, Inomata Y, Asonuma K, Egawa H, et al. Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in living related liver transplants. Transplantation 1998; 65:494–9.

Weusten JJAM, van Drimmelen HAJ, Lelie PN. Mathematic modeling of the risk of HBV, HCV, and HIV transmission by window-phase donations not detected by NAT. Transfusion 2002;42:537–48.

Available online at www.sciencedirect.com
ScienceDirect
Hepatology Research 36 (2006) 255–258
HEPATOLOGY RESEARCH
http://www.elsevier.com/locate/hepres

Editorial

Immunoprophylaxis of perinatal infection with hepatitis B virus on the national scale

Worldwide, an estimated 350 million people are persistently infected with hepatitis B virus (HBV). There are two routes for establishing the HBV carrier state. One is mother-to-baby transmission, and the other is horizontal transmission during the infancy. Often referred to as "vertical", by far the most mother-to-baby transmission of HBV is perinatal. The authentic vertical transmission *in utero* occurs in merely 3–5% of babies at high risk (*vide infra*). Theoretically, therefore, 95–97% of mother-to-baby transmission of HBV can be prevented after birth, provided that proper immunoprophylaxis is performed carefully and thoroughly. Chances for HBV persistence after horizontal infection decrease *pari passu* with increasing ages at infection [1]; 35% at 3 years, 26% at 5 years, 16% at 10 years and 11% at 15 years. HBV infection acquired during early infancy tends to continue during the rest of life, and can cause fatal liver disease in approximately 15–20% of them.

Not all babies born to mothers carrying HBV develop persistent infection. Inasmuch as HBV levels in the maternal circulation influence perinatal infection of their babies, there would be the threshold above which transmission occurs and below which it does not. In 1972, Magnius and Espmark [2] discovered hepatitis B e antigen (HBeAg) in sera of hemodialysis patients infected with HBV; they are known as highly contagious sources. Since then, HBeAg has been used as a marker of high infectious activity for the serum containing hepatitis B surface antigen (HBsAg) in many clinical and epidemiological settings. It was quite natural to assume that carrier mothers with HBeAg in serum are more likely to transmit HBV to their babies, and the outcome was amazing [3]. HBV infection persisted in all 10 babies and their 10 elder siblings born to carrier mothers with serum HBeAg, in sharp contrast to no persistent infections developing in any of 7 babies and their 3 elder siblings born to carrier mothers who possessed the antibody against HBeAg (anti-HBe) in serum.

Instigated by these clear-cut results, passive and active immunoprophylaxis of babies born to HBeAg-positive carrier mothers was planned, evaluated in field studies and escalated to the national scale in 1986 [4,5]. The immunoprophylaxis could protect 462 of the 494 (93.5%) babies, who were born to carrier mothers with serum HBeAg, from developing persistent HBV infection [5]. Since then, the national program is estimated to have protected approximately 95% of more than 4000 babies at risk from persistent HBV infection in Japan annually [6].

The initial immunoprophylaxis of perinatal HBV infection involved two doses of HBIG (50 IU each) given intramuscularly at birth and 2 months thereafter, and three doses of plasma-derived HB vaccine (5 μg per shot) at 2, 3 and 5 months after birth. HBIG given as soon as possible after birth is reasonable, because some neonates may not fully respond to HB vaccine by raising anti-HBs soon enough to escape perinatal infection. Furthermore, HB vaccine is postponed to 2 months after birth, thereby obviating any untoward effects that might be induced by vaccination at birth. Lo et al. [7] have clearly demonstrated the superiority of passive and active immunization, in comparison with vaccine alone (three doses at 2, 6 and 10 weeks after birth), in preventing perinatal HBV infection. In babies born to HBeAg-positive carrier mothers, perinatal transmission occurred in 24% (9/38) of those receiving vaccine alone, 11% (4/36) of those with vaccine plus one shot of HBIG (50 IU at birth) and only in 5% (2/38) of those with vaccine plus two shots of HBIG (again at 4 weeks).

It has been controversial if such additive effects of HBIG to plasma-derived vaccine, on preventing perinatal HBV transmission, hold true for recombinant vaccine engineered in yeasts [8,9]. In this issue of *Hepatology Research*, Kabir et al. [10] compared the efficacy of recombinant vaccine alone (three 10-μg doses) against that of vaccine plus HBIG (0.5 ml [units unspecified]) in Iranian babies born to mothers carrying HBV. HBsAg turned positive less frequently in babies receiving vaccine plus HBIG than those with vaccine alone (3.6% [2/55] versus 12.6% [15/119]). Thus, the advantage of combining HBIG with plasma-derived vaccine seems to have borne out with recombinant vaccine, also. This view

1386-6346/$ – see front matter
doi:10.1016/j.hepres.2006.10.001

goes along with the administration of HBIG to babies of carrier mothers with HBeAg and/or high-titer HBsAg in Taiwan [11] and all babies born to HBsAg-positive mothers in the United States [12], along with three doses of recombinant vaccine.

Their assertion would have been much more persuasive, however, had they focused on carrier mothers with serum HBeAg; it was detected only in 18.8% (9/48) and 15.2% (12/79) mothers, respectively, of babies with and without HBIG. Babies born to carrier mothers without serum HBeAg are much less likely to become chronically infected with HBV than those born to mothers without it [3]; such babies would most likely have escaped persistent HBV infection even without vaccination, with or without HBIG. Another issue of some concern, as the authors admit [10], is a high frequency of antibody to hepatitis B core (anti-HBc) detected in 38.7% (12/31) and 42.9% (21/49), respectively, in babies with and without HBIG. Hence, not all anti-HBs responses in the studied babies would have been raised by vaccination. Their results stand at variance with a marked decrease in the frequency of anti-HBc in Japanese children with anti-HBs, from 76.7% (23/30) before the national immunoprophylaxis to 9.0% (6/67) after it [4].

Fig. 1 illustrates the strategy for national immunoprophylaxis that may differ regionally depending on the main route of infection (perinatal or horizontal) and sizes of HBV reservoir in the community (prevalence of HBsAg in the general population) in each country.

(I) Selective immunoprophylaxis of babies born to HBeAg-positive carrier mothers has worked in Japan, because the prevalence of HBsAg was sufficiently low, and these babies were the principal source of horizontal HBV transmission to their siblings and playmates during the infancy. In actuality, the prevalence of HBsAg decreased from 0.75% (78/10,437) in Japanese children born before immunoprophylaxis to 0.04% (12/32,049) in those after it [4]. The improvement in sanitary conditions and implementation of disposable syringes and needles, as well as gain in economics, would have been prerequisite to such a remarkable decrease.

(II) In hyperendmic areas in Asia, typified by Taiwan, preventing perinatal transmission *per se* (started since 1984) did not contain HBV infection efficiently. A good part of horizontal infection was transmitted from diverse infectious sources lurking in the community densely contaminated with HBV, other than children of HBeAg-positive carrier mothers. In this setting, herd vaccination of all newborns was necessary (begun since 1987), in addition to selective passive and active immunoprophylaxis on high-risk babies. Combined with catch-up vaccination expanded from preschool children to adults during 1987 through 1990 [13], a great achievement has been gained in decreasing *de novo* HBV infection during the past 20 years in Taiwan.

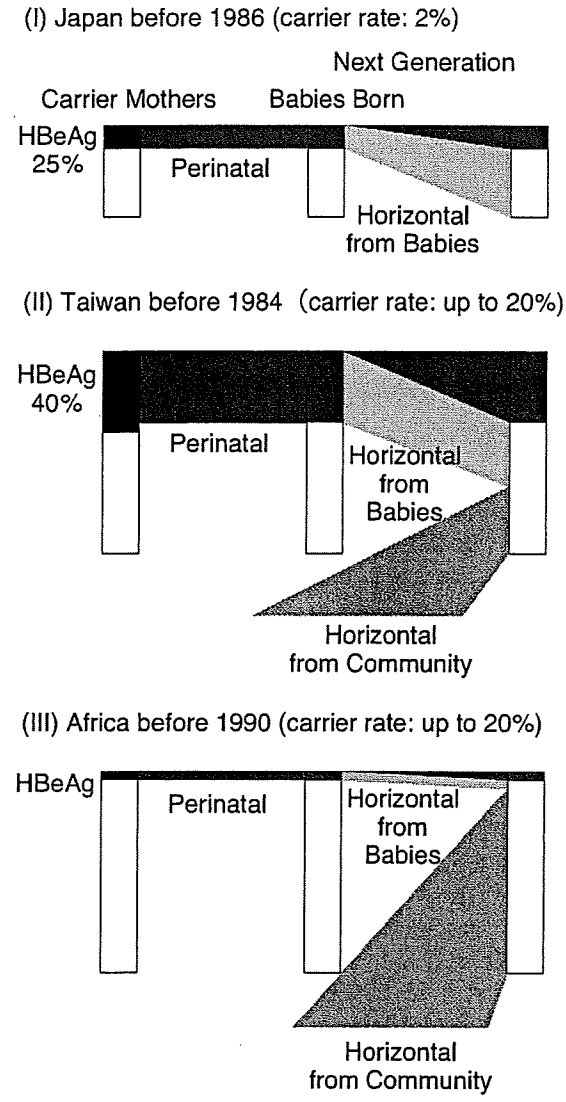


Fig. 1. Perinatal and horizontal transmissions for establishing the HBV carrier state. In the steady state of HBV infection in which the frequency of HBsAg remains constant over generations, the role of perinatal HBV transmission can be assessed by the prevalence of HBeAg in carrier mothers expecting babies. The impact of perinatal transmission is influenced by the size of total infection, as well. (I) In countries where the prevalence of HBeAg is moderate and the size of total infection small, babies born to HBeAg-positive carrier mothers are the main source of horizontal infection to their siblings and playmates. (II) In countries with high prevalence of HBeAg in carrier mothers and large size of total infection, horizontal infection in the infancy is acquired not only from babies with perinatal infection, but also from other sources lurking in the community. (III) In countries where perinatal infection from HBeAg-positive carrier mothers is rare, most infant HBV infections are contracted horizontally from sources other than babies born to such mothers. Examples are shown for Japan, Taiwan and Africa before the national immunoprophylaxis was started.

(III) The situation is much different in Africa, where the prevalence of HBsAg is high. Seroconversion to anti-HBe occurs very early in life due to inefficient translation of HBeAg inherent to subgenotype Aa of genotype A prevalent there [14]. This would be responsible, at least in part, for rare perinatal HBV

infection in Africa [15,16]. Horizontal transmission of HBV from the heavily polluted community is the most frequent route for establishing carrier state there. Preventing perinatal HBV transmission, therefore, does not have much impact in Africa. Mass vaccination and catch-up vaccination are reasonably indicated there; they may be realistic in developing countries, saving the costs for screening pregnant mothers for HBsAg and testing positive ones for HBeAg.

(IV) Dynamics of HBV infection are distinct from any of the above three in Northern Europe where the prevalence of HBsAg is merely 0.1% in the general population. Preventing perinatal infection would not be efficient for containing HBV, although it is recommendable [17]. Most HBV infections are contracted by adolescents and young adults primarily through promiscuous sexual contacts and sharing syringes and needles for injecting illicit intravenous drugs. Catch-up vaccination is indicated for individuals committing high-risk behaviors, especially because viral persistence is not infrequent after the adulthood infection with subgenotype Ae of genotype A prevalent there [18,19].

It has to be pointed out that environments can shift among the above four with time. Japan at present is a good example. With successful passive and active immunoprophylaxis of babies born to HBeAg-positive carrier mothers, the prevalence of HBsAg has decreased tremendously in adolescents born after 1986 [4,5]. It is worried, however, that HBV infection with subgenotype Ae is increasingly coming to the fore among young adults having promiscuous sexual contacts [20]. Epidemiologists and hepatologists would need to work out reasonable and effective measures to cope with evolving circumstances.

As of 1995, passive and active immunoprophylaxis was extended to cover babies born to carrier mothers without serum HBeAg; the second dose of HBIG in them is optional. At the same time, the cost of immunoprophylaxis was transferred from the government to health insurance policies. Possibly as the results of this, close follow-ups for anti-HBs responses in babies receiving immunoprophylaxis have become difficult in Japan. It is afraid that only 80% or less of high-risk babies have been protected since these changes. There is a pressing need in Japan to re-evaluate the current efficacy of immunoprophylaxis on high-risk babies, and take necessary measures for regaining the 95% goal once achieved by steady and concerted efforts.

References

[1] Edmunds WJ, Medley GF, Nokes DJ, Hall AJ, Whittle HC. The influence of age on the development of the hepatitis B carrier state. Proc Biol Sci 1993;253:197–201.

[2] Magnius LO, Espmark JA. New specificities in Australia antigen positive sera distinct from the Le Bouvier determinants. J Immunol 1972;109:1017–21.

[3] Okada K, Kamiyama I, Inomata M, Imai M, Miyakawa Y, Mayumi M. e antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. N Engl J Med 1976;294:746–9.

[4] Koyama T, Matsuda I, Sato S, Yoshizawa H. Prevention of perinatal hepatitis B virus transmission by combined passive-active immunoprophylaxis in Iwate, Japan (1981–1992) and epidemiological evidence for its efficacy. Hepatol Res 2003;26:287–92.

[5] Noto H, Terao T, Ryou S, et al. Combined passive and active immunoprophylaxis for preventing perinatal transmission of the hepatitis B virus carrier state in Shizuoka, Japan during 1980–1994. J Gastroenterol Hepatol 2003;18:943–9.

[6] Shiraki K. Perinatal transmission of hepatitis B virus and its prevention. J Gastroenterol Hepatol 2000;15(Suppl.):E11–5.

[7] Lo KJ, Tsai YT, Lee SD, et al. Combined passive and active immunization for interruption of perinatal transmission of hepatitis B virus in Taiwan. Hepatogastroenterology 1985;32:65–8.

[8] Hsu HM, Lee SC, Wang MC, Lin SF, Chen DS. Efficacy of a mass hepatitis B immunization program after switching to recombinant hepatitis B vaccine: a population-based study in Taiwan. Vaccine 2001;19:2825–9.

[9] Lolekha S, Warachit B, Hirunyachote A, Bowonkiratikachorn P, West DJ, Poerschke G. Protective efficacy of hepatitis B vaccine without HBIG in infants of HBeAg-positive carrier mothers in Thailand. Vaccine 2002;20:3739–43.

[10] Kabir A, Alavian SM, Ahanchi N, Malekzadeh R. Combined passive and active immunoprophylaxis for preventing perinatal transmission of the hepatitis B virus in infants born to HBsAg positive mothers in comparison with vaccine alone. Hepatol Res.

[11] Chen DS, Hsu NH, Sung JL, et al. A mass vaccination program in Taiwan against hepatitis B virus infection in infants of hepatitis B surface antigen-carrier mothers. JAMA 1987;257:2597–603.

[12] Mast EE, Margolis HS, Fiore AE, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. MMWR Recomm Rep 2005;54:1–31.

[13] Ni YH, Chang MH, Huang LM, et al. Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. Ann Intern Med 2001;135:796–800.

[14] Ahn SH, Kramvis A, Kawai S, et al. Sequence variation upstream of precore translation initiation codon reduces hepatitis B virus e antigen production. Gastroenterology 2003;125:1370–8.

[15] Botha JF, Ritchie MJ, Dusheiko GM, Mouton HW, Kew MC. Hepatitis B virus carrier state in black children in Ovamboland: role of perinatal and horizontal infection. Lancet 1984;1:1210–2.

[16] Marinier E, Barrois V, Larouze B, et al. Lack of perinatal transmission of hepatitis B virus infection in Senegal, West Africa. J Pediatr 1985;106:843–9.

[17] Polakoff S, Vandervelde EM. Immunisation of neonates at high risk of hepatitis B in England and Wales: national surveillance. BMJ 1988;297:249–53.

[18] Ozasa A, Tanaka Y, Orito E, et al. Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. Hepatology 2006;44:326–34.

[19] Suzuki Y, Kobayashi M, Ikeda K, et al. Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. J Med Virol 2005;76:33–9.

[20] Kobayashi M, Suzuki F, Arase Y, et al. Infection with hepatitis B virus genotype A in Tokyo, Japan during 1976 through. J Gastroenterol 2004;39:844–50.

Yuzo Miyakawa*

Miyakawa Memorial Research Foundation, Minami-Aoyama 2-19-8, Minato-Ku, Tokyo 107-0062, Japan

肝疾患治療における病診連携のありかた
—HCVをモデルとして—

田中純子・吉澤浩司
広島大学大学院医歯薬学総合研究科 疫学・疾病制御学講座／たなか・じゅんこ　よしざわ・ひろし

はじめに

2002年度から開始された「肝炎ウイルス検診」は，早くも今年で4年目を迎えた．

厚生労働省から公表された成績によれば，2002年度から2004年度までの3年間に，節目検診，節目外検診の両者を併せて合計5,372,501人がC型肝炎ウイルス検査を受診し，71,715人のC型肝炎ウイルス持続感染者（HCVキャリア）が見出されたとされている．

今後，この検診をより実効あるものとするためには，わが国の40歳以上の年齢層に潜在すると推定される約76万人のHCVキャリアを1人でも多く見出すこと，また，検査を受け，HCVキャリアであることがはじめてわかった人の病・医院受診率の向上を図ること，受診者が適切な健康管理，必要に応じた治療を受けることができる診療体制を早急に整備することなどが求められているといえる．

肝炎治療支援ネットワークの構築

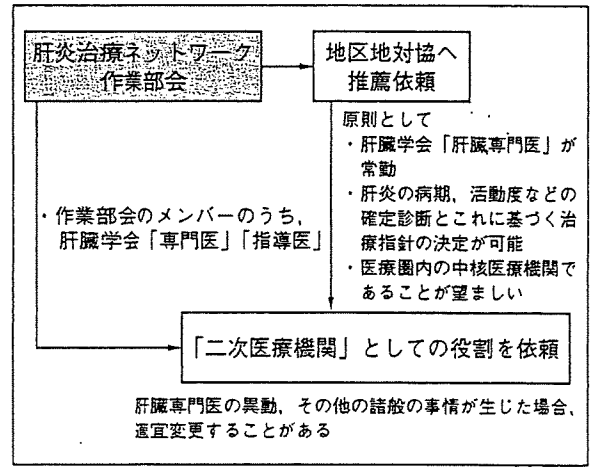
C型肝炎ウイルス検診が開始されてから3年の間に，岩手県[1]，茨城県[2]，石川県[3]，久留米市[4]，など，いくつかの地域において，それぞれの地域の特性を生かした形での肝炎ウイルスキャリアの健康管理，治療ネットワークの構築が試みられ，稼働しはじめている．

本稿では，広島県域において構築を試みた肝炎治療支援ネットワークについて述べてみたい．

広島県には，行政と医師会，そして大学の三者が一体となって地域保健の諸問題に取り組む，「地域保健対策協議会」（以下，地対協と記す）という組織が設置されている．その組織の中に1990年に新たに設置された「慢性肝疾患専門委員会」が中心となって，肝癌死亡率が全国でもつねに6位以内に位置する広島県における肝癌死亡率の減少を目指して，種々の活動を行ってきた．すなわち，県内の肝癌多発地域（某市）における死亡数（率）の推移に関する実態調査，県のパイロット事業の一環として地域住民検診へC型肝炎ウイルス検診を取り込むこと，高精度，簡便かつ安価なHCVキャリア発見のための検査の手順の作成，受診者への検査結果の通知方法およびHCVキャリア発見後の健康管理のあり方の検討などを行ってきた．また，献血を契機に見出されたHCVキャリアの病院初診時の肝病態やその後の経時的変化（治療効果や肝病態の進展度）を明らかにすることにより，HCVキャリアの健康管理から治療に至るまでの地区単位での病診連携のありかたについて検討してきた．

2002年度からは，「慢性肝疾患専門委員会」の中に新たに「肝炎治療支援ネットワーク作業部会」（以下，作業部会と記す）を設け，検診で発見された肝炎ウイルスキャリアの事後の健康管理を組織的に行うシステムの構築を試み，2003年4月から稼働させはじめている[5]．

HCVキャリアの組織的な健康管理を行うため



（広島県地対協　慢性肝疾患対策専門委員会）
図1　「二次医療機関」の任務依頼まで

●いくつかの地区において，肝炎ウイルスキャリアの健康管理，治療ネットワークの構築が試みられている．
●広島県では，医師会，行政，大学の三者が一体となって肝炎治療支援ネットワーク作りを勧めてきた．

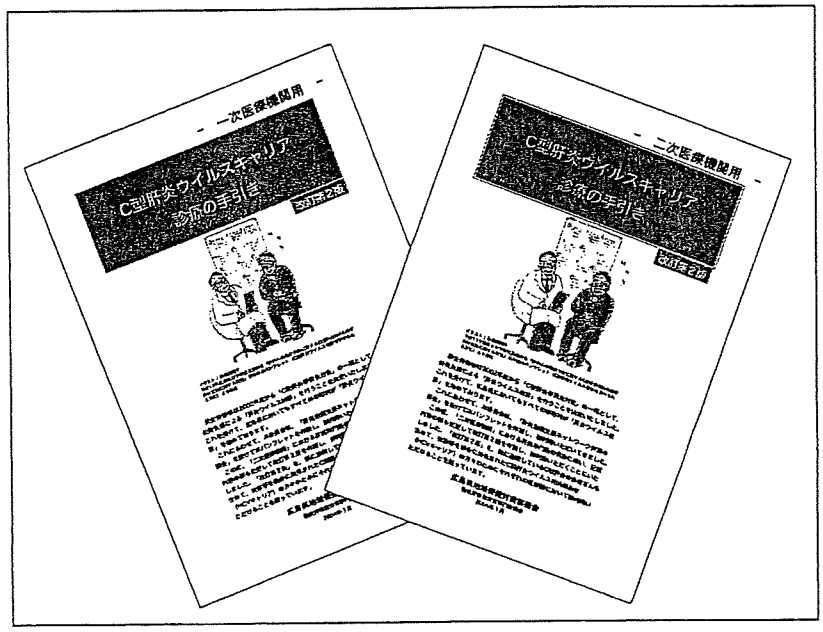


図2　C型肝炎ウイルスキャリア診療の手引き

には，かかりつけ医(一次医療機関)と肝臓専門医(二次医療機関)との連携，協力が不可欠である．作業部会では，県下の7つの二次医療圏に設置されているそれぞれの地区の地対協(地区地対協)に二次医療機関の推薦を依頼し，県医師会の理事会での承認を経て，合計37の病・医院に二次医療機関としての役割を依頼することとし，病・医院名および当該病・医院の肝臓専門医の氏名を公表した．二次医療機関の選定には，原則として，1)日本肝臓学会が認定した「肝臓専門医」が常勤していること，2)肝炎の病期，活動度などの確定診断とこれに基づく治療指針の決定が可能であること，3)当該二次医療圏内の中核医療機関であることが望ましいことなど，を条件とした(図1)．

肝炎治療支援ネットワークの実際●

1. 「一次医療機関」と「二次医療機関」の役割分担

作業部会では，肝炎治療支援ネットワークが円滑に運用されるよう，「一次医療機関」と「二次医療機関」の担当医に対して，それぞれの役割分担を理解してもらうための要点を具体的にまとめた手引書，「C型肝炎ウイルスキャリア診療の手引き」(一次医療機関用，二次医療機関用)を作成し(図2，3，4)，地対協事務局を通じて県下の内科系の医師会員全員に配布した．またC型肝炎に対する知識の共有を図るために，「HCVとC型肝炎の知識」[6]を各病院・医院に配布した．

「一次医療機関」用の手引きには，HCV検診は実施主体が市町村であることから，市町村の担当者が事後のフォローアップも含めて，直接関与することを強調して記載した．また，経過観察を行う際の検査項目を記載し，定期的に二次医療機関への受診を勧め，肝炎の活動度，病期の再確認を行って健康管理，治療方針を決めることが大切であることを記述した(図3)．

「二次医療機関」用の手引きには，紹介された患者は精査の上，以後の健康管理，治療方針を記載

1. C型肝炎ウイルス抗体(HCV抗体)陽性者は？

HCV抗体慢性者は，過去にHCVに感染して治癒した後の人(感染既往者)と，現在HCVに感染している人(そのほとんどはHCVキャリア)とに分けられます．
今回の検診は，HCVキャリアを発見するための検査が行われています．したがって，今回の検診の結果をもとに「健康管理手帳」を持参して受診される患者さんのほとんどはHCVキャリアです．

2. C型肝炎ウイルス持続感染者(HCVキャリア)とは？

C型肝炎ウイルス(HCV)が肝臓の中に住みついている(持続感染している)人をHCVキャリアと呼びます．
検診などの機会に「HCVに感染している」ことがはじめてわかった人のほとんどはHCVキャリアであることがわかっています．
HCVキャリアを放置した場合，肝がんに進展する場合もあるので注意が必要です．

3. HCVキャリアとC型慢性肝炎との関係は？

HCVキャリアの肝生検組織を調べてみると，程度の差はあるものの，ほとんどすべての例の肝臓に慢性の炎症(慢性肝炎)が認められます．
HCVキャリアは，慢性の炎症(慢性肝炎)の程度により，定期的に検査を行い，経過を診ることから始めてよい人と，直ちに治療を始める必要がある人とに分けられます．

4. HCVキャリアの経過観察の手順は？

初診時の理学的所見，検査値等に異常を認めない場合でも，引き続き1ヶ月に1回の頻度で2～3回検査を行ない，検査結果を受診者が持参する「健康管理手帳」に記入の上，二次医機関へ紹介して下さい．
紹介先の二次医療機関から，「定期的な検査による経過観察」の返事を得た場合は，以後の検査は2ヶ月に1回程度とし，検査結果をその都度「健康管理手帳」に記入して，患者さんを少なくとも年に1回程度は二次医療機関へ紹介するなどして，精査を勧めて下さい．
初診時の理学的所見，検査値などに異常を認めた場合は，検査結果等を「健康管理手帳」に記入の上，患者さんを直ちに二次医療機関へ紹介し，以後は二次医療機関との連携の下に治療，経過の観察等を行ない，定期的に病期の判定，治療方針の決定等を行なって下さい．

5. HCVキャリアの初診時の検査項目は？

初診時，および経過観察時に，最低限下記の項目を検査して下さい．

1. ALT(GPT)，AST(GDT)
2. ZTT
3. LDH
4. ALP
5. γ-GTP
6. 末梢血検査，(血算，血小板)
7. HCV RHA量の測定(アンプリコアモニター)*1
8. HCVのセロタイプの決定*2

*1 この方法により，HCV RNAが陰性と判定された場合でも，HCV RNA量は変動することが多く，この方法による検出感度未満のHCV RNAが存在する場合がありますので，経過観察は続行して下さい．
HCVキャリア状態からの離脱(完全治癒)が起こっているか否かの判断は，二次医療機関の判定にゆだねてください．

*2 インターフェロン治療の適応を決める等の際に必要な感染ウイルス株を決める簡便検査法ですので，初診時に1回だけ検査して下さい．

6. 市町村との連携は？

「肝炎ウイルス検診」は，各市町村を実施主体とする公費負担による事業であることから，検診等を契機に発見されたHCVキャリアの医療機関への受診の有無を把握するなど，各市町村は検診受診者に対する事後の保健指導を行なうことが義務づけられています．患者さんが受診した場合には，下記の要領で連絡をしてください．
HCVキャリアの方が受診した際には，「健康管理手帳」に添付の返信用はがきに，受診日，担当医氏名を御記入の上投函するか，患者さんに投函してもらうようにして下さい(患者さんの氏名を記入する必要はありません)．

(広島県地対協　慢性肝疾患対策専門委員会)

図3　C型肝炎ウイルスキャリア診療の手引き「一次医療機関」用

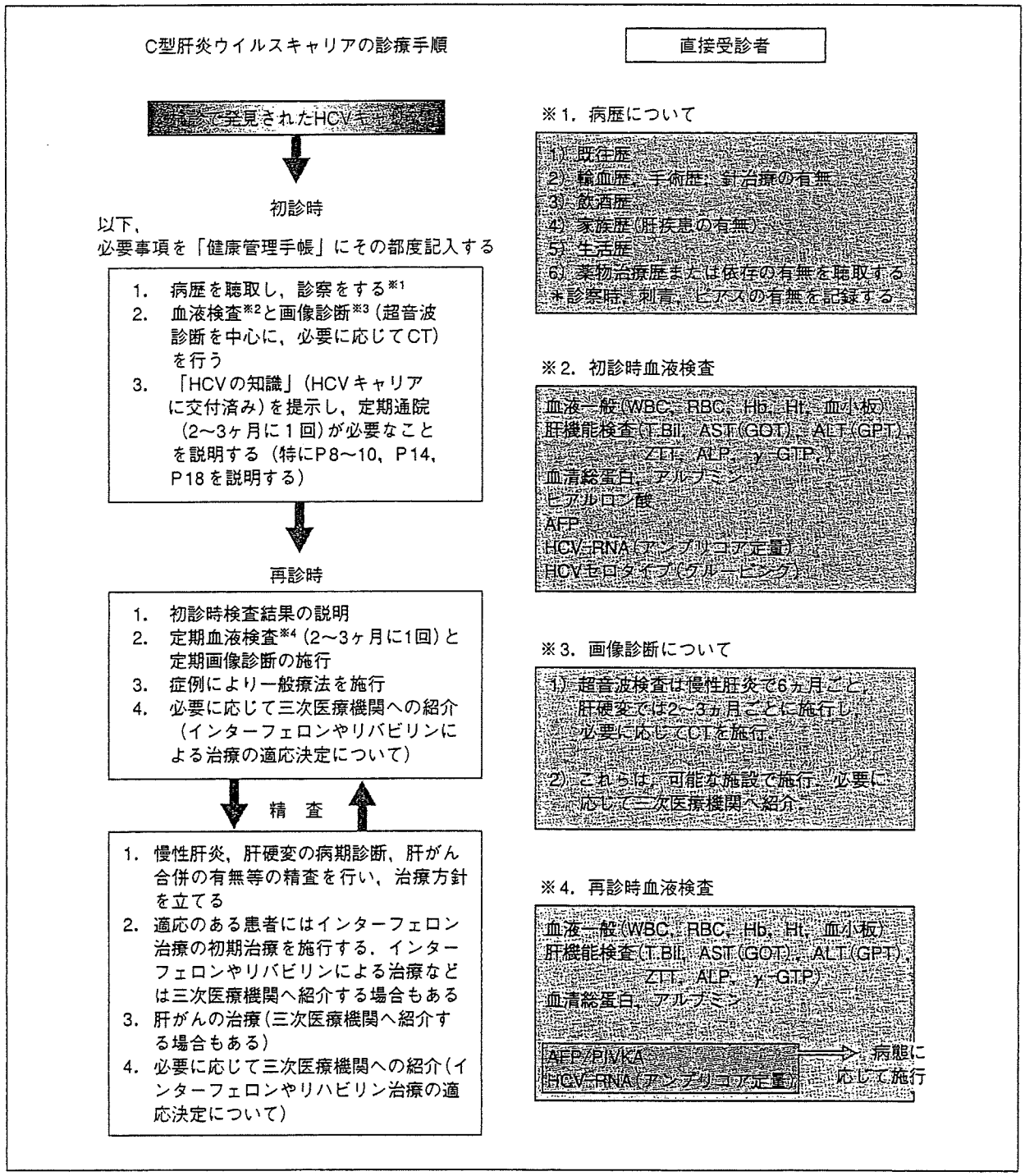


図4　C型肝炎ウイルスキャリア診療の手引き「二次医療機関」用

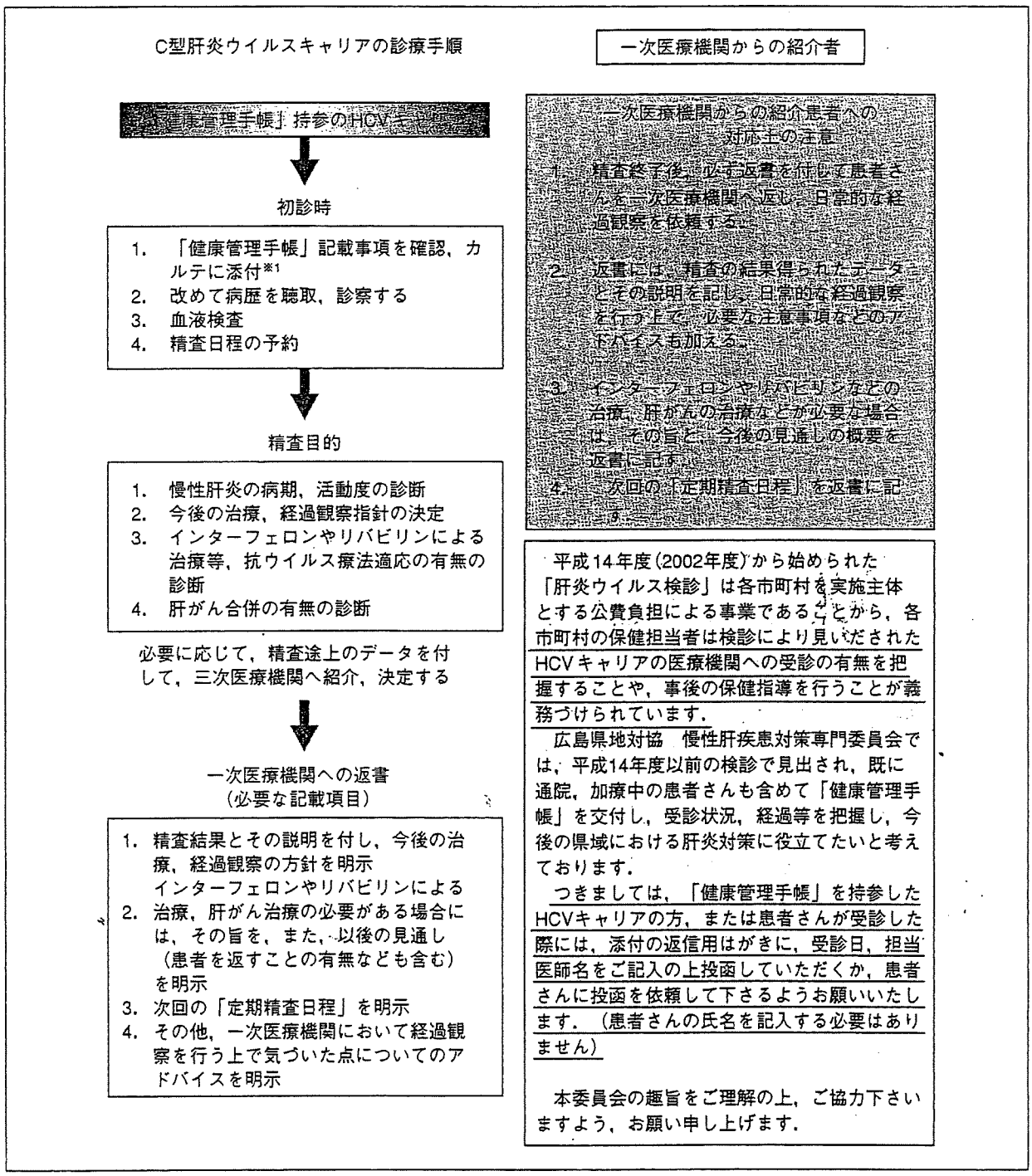


図4 つづき (広島県地対協 慢性肝疾患対策専門委員会)