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A novel hepatitis B virus surface antigen immunoassay as sensitive as hepatitis B virus nucleic acid testing in detecting early infection

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BACKGROUND: The aim was to considerably enhance the sensitivity of hepatitis B virus (HBV) surface antigen (HBsAg) detection and investigate whether the window period for HBV detection could be reduced.

STUDY DESIGN AND METHODS: A high-sensitivity chemiluminescent enzyme immunoassay (CLEIA) was developed for quantitative HBsAg detection by a combination of monoclonal antibodies, each one for a specific epitope of HBsAg, and by improving the conjugation technique. The sensitivity of the assay was compared with that of the existing chemiluminescent immunoassay (CLIA). Commercially available seroconversion panels and samples of HBV-infected chimpanzees were tested with the developed prototype to assess whether the window period for HBsAg detection could be reduced to that for DNA detection.

RESULTS: Compared to the existing CLIA, the CLEIA prototype detected HBsAg with approximately 230-fold higher sensitivity and showed a reduced window period. HBsAg detection by the CLEIA prototype and HBV DNA detection by polymerase chain reaction (PCR) occurred simultaneously. The mean time for the CLEIA prototype to first detect HBsAg was approximately 17.4 days less than that for the existing systems. Further, CLEIA prototype enabled HBsAg detection even in anti-HBs–positive seroconversion samples. In the inoculated chimpanzees the HBsAg and HBV DNA became detectable simultaneously and concentrations increased in parallel, whereas HBsAg remained detectable longer than HBV DNA in the declining phase of viremia.

CONCLUSION: The CLEIA prototype yielded results comparable with those of HBV DNA PCR. This novel high-sensitivity assay may be useful for early detection of HBV infection and monitoring patients with a history of infection.

Hepatitis B virus (HBV) infection is one of the main causes of chronic hepatitis. It is estimated that more than 2 billion people worldwide are infected with HBV, of which approximately 400 million people are chronically infected.[1-4] HBV is transmitted by exposure to infectious blood, for example, via accidental injection and transfusion of blood or sexual contact and from the mother to a newborn infant. The development of a persistent HBV infection depends on the age of the infection and immune status of the host.[1,3-12] It is reported that 10 to 30 percent of individuals persistently infected with HBV develop liver cirrhosis and hepatocellular carcinoma. HBV infection causes up to 80 percent of liver cancers.[1,3,4,13-15]

HBV is a double-stranded DNA virus, composed of a nucleocapsid core coated with an envelope containing the

ABBREVIATIONS: CLEIA = chemiluminescent enzyme immunoassay; CLIA = chemiluminescent immunoassay; ER = endoplasmic reticulum.

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Experiments using chimpanzees had been performed before 2006 and the ethical approval for each experiment had been gained which was applied according to the form of the facilities.

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surface antigen (HBsAg). The diagnosis of HBV infection involves the detection of specific antigens and/or antibodies such as HBsAg, antibody to HBsAg (anti-HBs), antibody to HBV core antigen (anti-HBcAg), hepatitis Be antigen (HBeAg), and antibody to HBeAg. HBV DNA is considered to be followed after a few weeks by HBsAg during HBV infection. Enzyme-linked immunoassay (ELISA) is currently used in the commercial HBsAg detection assays[16-19] because of its simplicity and cost-effectiveness.

Despite the fact that many countries perform HBV DNA and HBsAg screening tests, posttransfusion HBV infection continues to occur.[20-25] This is because a relatively long infectious window period remains before HBV DNA and HBsAg become detectable in serum.[19-22,24-30] HBV has been reported to exist in the serum in distinct morphologic forms, the most abundant of which are small or tubular HBsAg particles.[1,26] Empty HBsAg particles are composed of HBsAg and do not contain DNA. The number of these particles is 10,000 to 1,000,000 more than the number of Dane particles, which are infectious HBV particles containing HBV DNA. Because of the large number of empty HBsAg particles, the potential for theoretical detection of HBsAg may be greater.

Although the sensitivity of the HBsAg assay has been improved, reduction in the window period such that it is similar to that of DNA detection remains difficult.[18,19,24,25,27-29,31] The window period for HBV detection has been estimated to be 35 to 76 days by individual sample nucleic acid testing (NAT), 41 to 90 days by minipool NAT, and 50 to 97 days by high-sensitivity HBsAg chemiluminescent immunoassay (CLIA).[28,30] In this study, an unprecedented high-sensitivity prototype was developed and evaluated. The sensitivities of the newly developed prototype and the existing detection systems were compared. To investigate the prototype's ability to detect HBV infection in the early phase, we used the developed prototype to compare the time of appearance of HBsAg and HBV DNA in seroconversion panels. Subsequently, the detection ability of the developed prototype was compared with the detection abilities of the DNA detection and existing HBsAg detection systems.

MATERIALS AND METHODS

Samples

Seroconversion panels were purchased from BioClinical Partners, Inc. (Franklin, MA; Panels 6277, 6278, 6279, and 6281), and Boston Biomedica, Inc. (West Bridgewater, MA; Panels PHM 902, PHM 903, PHM 908, PHM 909, PHM 910, PHM 911, PHM 912, PHM 914, PHM 915, PHM 916, PHM 919, PHM 920, PHM 922, and PHM 935B). The HBsAg sensitivity panel PHA 808 (Boston Biomedica, Inc.) was used for comparing the in-house and international units. The ninth sample obtained from PHA 808 was diluted 5- to 100-fold with a pooled negative serum sample and used for the dilution study. Individual samples that were negative for all viral markers were purchased from ProMedex, Inc. (Flushing, NY).

Chimpanzees

Sequential samples obtained from six chimpanzees infected with HBV reported previously were tested.[30] Chimp 246 was inoculated intravenously with fresh-frozen plasma units from blood donors acutely infected with genotype A (6.9×10^4 copies/mL), and Chimp 272, with genotype C (5.3×10^5 copies/mL). Chimps 279 and 280 were inoculated with pre–acute-phase plasma of Chimp 246, and Chimps 269 and 285 were inoculated with pre–acute-phase plasma of Chimp 272. After the inoculation, serum samples were obtained at intervals of 2 to 7 days in the early phase of infection and as required. The collected samples were assessed for HBsAg by the chemiluminescent enzyme immunoassay (CLEIA) prototype and for HBV DNA by polymerase chain reaction (PCR; TaqMan, Roche Diagnostics KK, Tokyo, Japan) with a sensitivity of 100 copies per mL.

Recombinant HBsAg

Recombinant HBsAg for the in-house standard was purchased from HyTest Ltd. (Turku, Finland). The recombinant HBsAg was serially diluted to produce the in-house standard. The concentration was expressed in international units (IUs; IU/mL or mIU/mL) by calibrating it against the World Health Organization (WHO) reference standard. The measured unit values were compared with the values measured by the existing quantitative HBsAg detection system (ARCHITECT, Abbott Laboratories, Abbott Park, IL), which was based on CLIA method.

Monoclonal antibodies

Monoclonal antibodies (MoAbs) to HBsAg were obtained from established hybridomas by the following method. The recombinant antigen was mixed with an equal volume of Freund's adjuvant, and a dose of 10 to 20 μg of the resultant mixture was intraperitoneally administered to 4- to 6-week-old BALB/c mice. A booster was intraperitoneally administered every 2 to 4 weeks, and a final immunization dose of 10 μg was injected into the caudal vein. After 3 days, splenocytes were obtained from the immunized mice and fused with myeloma cells. The fusion cells were suspended in RPMI 1640 containing 10 percent fetal bovine serum, hypoxanthine, aminopterin, and thymidine and plated onto a 96-well cell culture plate. After approximately 10 days of culturing, when only the hybridomas were proliferated, clones producing the required antibody were selected.

Development of the CLEIA prototype for detecting HBsAg

A CLEIA prototype was constructed for testing the performance of the high-sensitivity HBsAg assay. This sandwich assay utilizes a 96-well microtiter plate (FluoroNunc Module Maxisorp surface; Nunc, Roskilde, Denmark) coated with MoAbs that recognize HBsAg. To detect HBsAg more effectively, MoAbs were selected for ELISA, since each antibody recognized a different HBsAg epitope. Three clones were used for solid antibodies, each of which recognized the determinant "a" as the constructive epitope, amino acids 111 to 130, and amino acids 31 to 50, respectively. For tracer antibodies, two clones were used, each of which recognized the determinant "a" as the constructive epitope in the different region of the solid antibody and amino acids 51 to 69, respectively. The conjugate formed comprised anti-HBs MoAbs labeled with alkaline phosphatase (Roche Diagnostics, Mannheim, Germany), which were linked to some mass molecules, for example, dextran, beta-galactosidase, and polymerized albumin by the modified method reported;[32] these alkaline phosphatase–labeled antibodies enhanced the signals, avoiding aggregation and minimizing the detection limit. In the case using albumin, albumin (32 mg) was mixed with 100 µL glutaraldehyde to produce the polymerized albumin. Next, 1.5 mol per L Tris buffer was added to stop the reaction, and it was incubated for at least 3 hours. The product was purified by gel permeation, and it was used as the linker between the enzyme and the antibody. The antibody was used after pepsin digestion to remove the Fc region, treated with 15 mmol per L mercaptoethylamine for 90 minutes, and conjugated with the polymerized albumin-linked enzyme using *N*-(6-maleimidocaproyloxy)succinimide (Dojindo Laboratories, Kumamoto, Japan). The product was purified by gel permeation and stored with preservative and carrier bovine serum albumin before use. For analyzing HBsAg in the serum or plasma, 75 µL of the sample and 25 µL of the reaction buffer (containing 100 mmol/L Tris buffer supplemented with 1% Triton X-100, 1% sodium dodecyl sulfate, and 1% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; pH 7.2) were added to the antibody-coated microwells and incubated at 37°C for 60 minutes. After the wells were washed six times with a washing buffer (phosphate-buffered saline containing 0.05% Tween 20), 100 µL of alkaline phosphatase–conjugated anti-HBsAg MoAbs was added to the wells and incubated at room temperature for 30 minutes. After incubation, the microwells were washed again six times and incubated with 100 µL CDP-Star with Emerald II (Tropix, Inc., Bedford, MA). After incubation at room temperature for approximately 15 minutes, the relative light units of the microwell samples were measured. The results were expressed in IU values, which were determined according to the in-house standard curve.

Assessment of the CLEIA prototype

The selected HBsAg-positive samples were diluted with pooled normal serum, and the dilution linearity was assessed. On the basis of the in-house standard curve, linearity was defined relative to the calculated amount of HBsAg.

RESULTS

Performance of CLEIA for HBsAg detection

The amount of recombinant HBsAg was expressed in IU by calibrating it against the WHO reference standard. The dilution series of the recombinant HBsAg were analyzed for linearity and used as the in-house standard. The lowest concentration of the in-house standard was 4.6 mIU per mL with a signal-to-noise ratio of 22.5 (Fig. 1A). In the same assay, the analytical sensitivity of the CLEIA prototype was 0.05 mIU per mL. To confirm the consistency of the IU values, which was determined from the in-house standard values, the PHA 808 panel was tested. A high correlation was observed between the IU value determined from the in-house standard and that of the international standard mentioned in the data provided (Fig. 1B). The unit values of the existing quantitative HBsAg assay and those of the CLEIA prototype were also compared. One HBsAg-positive serum sample was determined to have a titer of 11.08 IU per mL according to the existing quantitative HBsAg CLIA; it was diluted with pooled normal serum and measured using both the existing CLIA and the CLEIA prototype. The sample was diluted serially from 5- to 3125-fold: the expected value was in the range of 2.2 to 3.5 mIU per mL, although the cutoff value of the existing CLIA was 50 mIU per mL. The data of the comparison between the existing CLIA and the CLEIA prototype are shown in Fig. 1C. The approximation formulas for the unit value were determined by the existing CLIA or the CLEIA prototype, and the expected unit values were $Y = 1.078X^{0.695}$ and $Y = 1.405X^{0.997}$, respectively.

Dilution linearity test

Three individual HBsAg-positive samples were diluted with pooled normal serum and assessed using the CLEIA prototype. Table 1 shows the dilution linearity of the CLEIA prototype in the range of 97.6 to 115.3 percent. The ninth sample of PHA808, which showed a value of 0.05 IU per mL according to the data provided, was also diluted for analyzing the dilution linearity at a low titer of HBsAg. The recovered values calculated using the dilution factor ranged from 96.4 to 113.8 percent (Table 1),

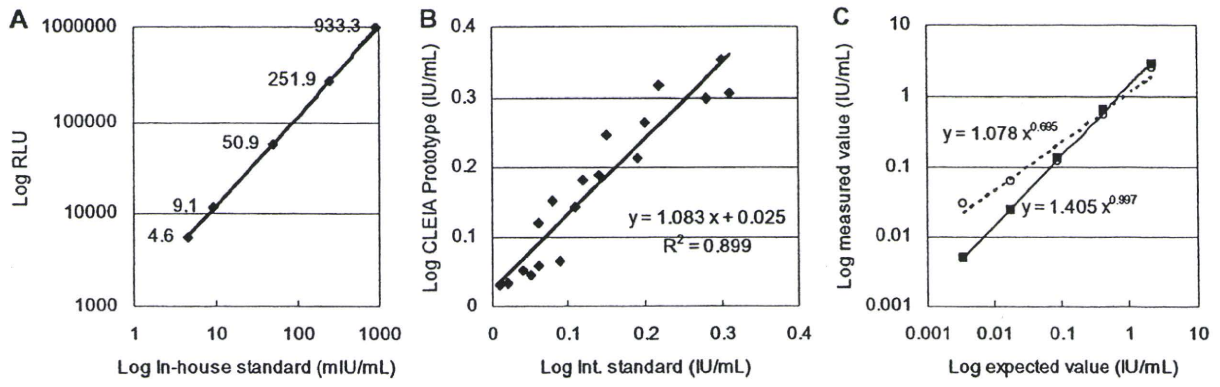

Fig. 1. The performance of the CLEIA prototype was evaluated and expressed in IU values. (A) The in-house standard (933.3-4.6 mIU/mL) determined using recombinant HBsAg was measured by the CLEIA prototype. (B) The concentrations of the HBsAg sensitivity panel PHA 808 (Boston Biomedica, Inc.) measured by the CLEIA prototype were compared with the provided data. The correlation coefficient is shown as $R^2 = 0.899$. (C) HBsAg-positive serum (11.08 IU/mL) was diluted with pooled normal serum and measured by the existing quantitative CLIA (○) and the CLEIA prototype (■).

TABLE 1. Dilution linearity for four HBsAg-positive samples

Sample	Dilution factor (DF)	Observed value (mIU/mL) × DF	Expected value (mIU/mL) × DF	Recovery rate (%)
HBsAg-positive 1*	Neat	35.0	35.0	100.0
	5	38.9		111.2
	25	37.2		106.4
HBsAg-positive 2*	Neat	67.8	67.8	100.0
	5	66.1		97.6
	25	78.2		115.3
HBsAg-positive 3*	Neat	264.7	264.7	100.0
	5	263.8		99.7
	25	281.0		106.2
No.9 from PHA808†	Neat	50.0	50.0	100.0
	5	51.2		102.4
	10	48.2		96.4
	20	61.4		122.7
	50	55.3		110.7
	100	56.9		113.8

* HBsAg-positive samples.
† The ninth sample obtained from the purchased panel PHA 808. The sample was calibrated against the international standard as 0.05 IU per mL by the panel vendor (Boston Biomedica, Inc.). The assay results were provided by the panel vendor.

while all the five points were below the cutoff value of the existing CLIA.

Normal sample distribution measured by the CLEIA prototype

Using the CLEIA prototype, we measured 230 serum and 273 plasma samples obtained from normal individuals. The results of the 503 normal samples were analyzed to set the cutoff value of the CLEIA prototype (Fig. 2). The mean and mean + 10 standard deviations (SDs) were 0.10 and 0.22 mIU per mL, respectively, and the cutoff point was set at 0.22 mIU per mL.

Performance in the commercially available seroconversion panels

A total of 18 commercially available seroconversion panels were tested using the CLEIA prototype. These panels were composed of serial specimens collected from individuals during a period of hepatitis B seroconversion. All the individuals became HBsAg-positive during the collection period, and two of the selected panels, namely, HBP 6281 and PHM 935B, were assessed as anti-HBs–positive according to the data supplied by the manufacturer. All panels were assessed as HBV DNA–positive before the appearance of detectable HBsAg when measured by the commercially available detection systems (data provided by the manufacturer). Serial specimens collected from 16 individuals during the hepatitis B seroconversion were tested using the CLEIA prototype. The detection time (days) for the CLEIA prototype was compared with that for the DNA detection and existing HBsAg detection systems (data provided). The mean number of days after which HBV DNA and HBsAg were first detected were 21.8 and 41.9 days, respectively, in the 16 panels; this implied a mean difference of 20.1 days (Table 2). The mean difference in the first HBsAg detection between the CLEIA prototype (24.5 days) and the existing systems (41.9 days) was 17.4 days. This showed that the CLEIA prototype was the most sensitive HBsAg detection assay compared with the other assays (data provided). The representative seroconversion panel with short sampling intervals was shown in Table 3. When

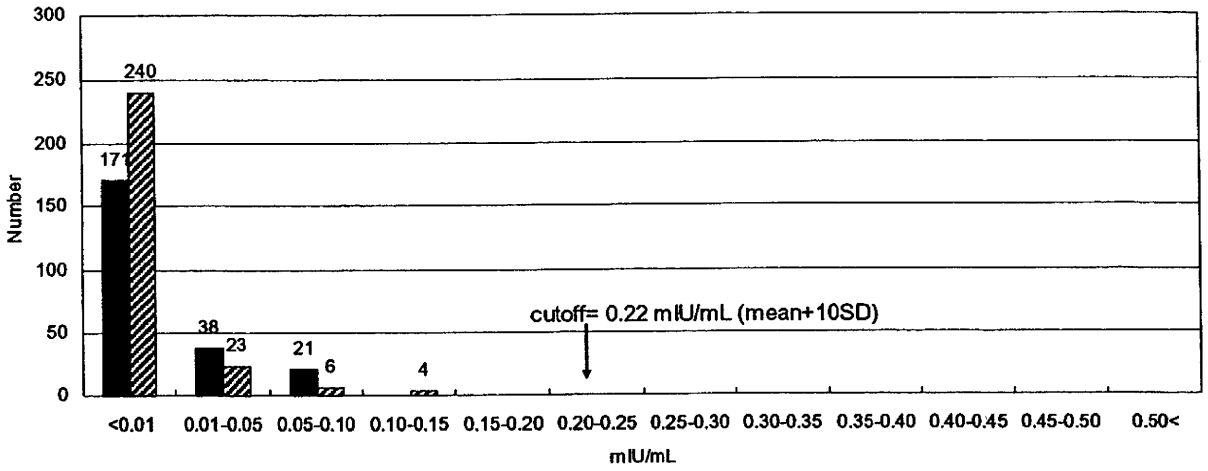

Fig. 2. The normal sample distribution was measured by the CLEIA prototype. To test the specificity of the CLEIA prototype, 230 serum samples (■) and 273 plasma samples (▨) obtained from HBV-negative individuals were measured. The highest values in the serum and plasma were 0.10 and 0.14 mIU per mL, respectively. The mean value in the 503 samples measured was 0.10 mIU per mL. The cutoff point was set at 0.22 mIU per mL as the mean + 10 SDs value.

TABLE 2. Comparison of the assay performances for HBV detection in seroconversion panels

	Days to detection from the first bleed			Window reduction by the CLEIA prototype compared with		
Panels	DNA	Existing HBsAg*	Prototype	Existing HBsAg detection system	DNA detection system	Earliest HBV detection
PHM 902†	57	71	57	14	0	DNA, prototype
PHM 903†	0	14	0	14	0	DNA, prototype
PHM 908†	0(+/–)	33	5	28	–5	DNA
PHM 909†	0	9	0	9	0	DNA, prototype
PHM 910†	18(+/–)	35	0	35	18	Prototype
PHM 911†	49	79	63	16	–14	DNA
PHM 912†	0	42	12	30	–12	DNA
PHM 914†	146	158	146	12	0	DNA, prototype
PHM 915†	0	33	0	33	0	DNA, prototype
PHM 916†	48	65	50	15	–2	DNA
PHM 919†	0(+/–)	19	0	19	0	DNA, prototype
PHM 920†	5(+/–)	26	26	0	–21	DNA
PHM 922‡	0	16	0	16	0	DNA, prototype
HBV 6277†	26	33	21	12	5	Prototype
HBV 6278†	0	12	0	12	0	DNA, prototype
HBV 6279†	0	26	12	14	7	Prototype
Mean	21.8	41.9	24.5	17.4	–2.7	

* The data provided for PHM 902 through PHM 922 (Boston Biomedica, Inc.) represent the results of an overnight assay performed by binding of the Auszyme MoAb to the HBsAg (Abbott Laboratories). The data for HBV 6277, HBV 6278, and HBV 6279 were provided by Zeptometrix Corp. (Buffalo, NY) and analyzed by PRISM HBsAg (Abbott Laboratories).
† The detection limit for the HBV DNA assay is 100 copies per mL.
‡ The detection limit for the HBV DNA assay is 10 copies per mL.

compared by signal-to-cutoff ratio, the CLEIA prototype showed approximately 110-fold higher sensitivity than PRISM (cutoff, 25 mIU/mL). In 8 of the 16 panels tested, HBsAg detection by the CLEIA prototype and HBV DNA detection by PCR (the limit of detection in the provided data was 100 copies/mL or less) occurred simultaneously. In 2 of the 16 panels tested, namely, PHM 910 and HBV 6277, the CLEIA prototype detected HBsAg earlier than PCR detected HBV DNA. In the remaining 6 panels, HBV DNA detection by PCR occurred earlier than HBsAg detection by the CLEIA prototype. The difference in the window period for HBV DNA and HBsAg detection was only 2.7 days.

The two panels that became anti-HBs–positive were also tested (Fig. 3). In PHM 935B, DNA was not detected (the limit of detection in the provided data was 400 copies/mL) in the late phase of infection. It was indicated that the PRISM HBsAg assay (Abbott Laboratories), which

TABLE 3. The result of HBV 6279

Panel	DNA PCR	HBsAg (S/CO) PRISM	HBsAg (S/CO) Prototype
HBV6279-01	278	0.36	0.6
HBV6279-02	NEG	0.33	1.7
HBV6279-03	Not tested	0.24	3.2
HBV6279-04	75.3	0.38	11.8
HBV6279-05	529	0.53	32.8
HBV6279-06	5110	1.63	107.8

S/CO = signal-to-cutoff ratio.

showed the highest sensitivity in the data provided, detected the 246th sample that had become anti-HBs–positive. The CLEIA prototype exhibited a higher sensitivity than PRISM; it detected all the samples in PHM 935B, even those in the anti-HBs–positive phase. In HBV 6288, DNA (the limit of detection in the provided data was 100 copies/mL) and HBsAg measured by the CLEIA prototype alone detected HBV in the first sample. In the late phase of HBV 6288, both DNA and HBsAg measured by PRISM did not detect HBV in the sample that became anti-HBs–positive (data provided). In contrast, the CLEIA prototype detected the last sample that was anti-HBs–positive.

Detection of HBsAg in chimpanzee HBV infectious models

The first detection day for Chimp 246 (genotype A) was Day 17 for HBV DNA at 120 copies per mL and Day 15 for HBsAg measured by the CLEIA prototype at 0.24 mIU per mL (Fig. 4A). In the late phase of infection, HBV DNA was detected at approximately 100 copies per mL by Day 334. In contrast, HBsAg persisted longer than HBV DNA, which was detected by Day 377. In Chimp 272 (genotype C), HBV DNA was detected at 300 copies per mL on Day 6 after inoculation, and HBsAg was detected by the CLEIA prototype on Day 10 from inoculation (Fig. 4B). In the latter phase of infection, HBV DNA was detected on 100 copies per mL at Day 189, and HBsAg measured by the CLEIA prototype was detected on Day 182. The first detection days of DNA measured by qualitative nested PCR and HBsAg measured by AxSYM (Abbott Laboratories) or the CLEIA prototype were compared in the samples from six chimpanzees (Table 4). The mean number of days which HBV DNA and HBsAg measured by AxSYM or the CLEIA prototype were 28.5, 56.7, and 38.2 days, respectively. The mean difference in the first HBsAg detection between AxSYM and the CLEIA prototype was 18.5 days. The difference in the window period for HBV DNA measured by qualitative PCR and HBsAg measured by the CLEIA prototype was 9.7 days.

DISCUSSION

NAT can detect a low-titer HBV infection. It is considered to be a superior technique for the early detection of HBV infection.[24,25,28] However, despite the regular use of minipool NAT for blood donor screening, the risk of posttransfusion HBV infection persists.[20-25] In this study, we developed an unprecedented high-sensitivity HBsAg detection prototype and investigated whether such an assay could reduce the risk of undesired HBV transmission.

For the development of the CLEIA prototype, we focused on the epitope that was the target region of the antibodies used for HBsAg detection. HBsAg is a four-transmembrane protein comprising 226 amino acid residues (amino acids 1-226). Although the transmembrane structure of HBsAg is not completely elucidated, it has been proposed that HBsAg is composed of an endoplasmic reticulum (ER) lumen region comprising amino acids at positions 1 to 11 from the N-terminal of HBsAg, a hydrophobic transmembrane region comprising amino acids at positions 12 to 28, a region inside the lipid bilayer comprising amino acids at positions 29 to 80, a hydrophobic transmembrane region comprising amino acids at positions 81 to 97, a hydrophilic ER lumen region comprising amino acids at positions 98 to 156, and two hydrophobic transmembrane regions comprising amino acids at positions 157 to 226.[33] A common determinant "a," which is used in the conventional methods for HBsAg detection, is contained in the amino acids at positions 110 to 156, particularly in the amino acids at positions 98 to 156 localized on the ER lumen side, that is, on the surface of the viral particle. It is indicated that the detection limit is improved when the MoAbs, each bound to a distinct epitope, are used in combination.[34] In this study, to detect the HBsAg more effectively, we used MoAbs that recognized HBsAgs localized inside the lipid bilayer, on the ER lumen side, and at the position of the common determinant "a." To enhance the effect of antibodies that recognize the inner epitope, detergents were added in the reaction buffer. It is confirmed that detergents were necessary for the reaction of such antibodies in the preliminary experiment. Detergents might help to exposure the epitope localized inside the lipid bilayer, and this is one of the characters of the CLEIA prototype. Furthermore, we tried to increase the detection limit by improving the tracer.[32] The improvement of the uniting ratio of the enzyme and antibody is the way to enhance the signal. However, especially for the high-molecular-weight molecule such as alkaline phosphatase, the high ratio conjugation often causes the aggregation. The aggregated tracers cause the high background and sometimes cause the low positive signal by covering the antigen-binding site of antibody with enzyme. In this report, we made the labeled tracer as a hydrophilic mass molecule and successfully prevented the aggregation. Although this is a simple method to improve sensitivity, which was only modified from the classical technique,[32] it can be applied to many types of conjugation.

The detection limit and cutoff value for the CLEIA prototype were investigated by standardizing the unit

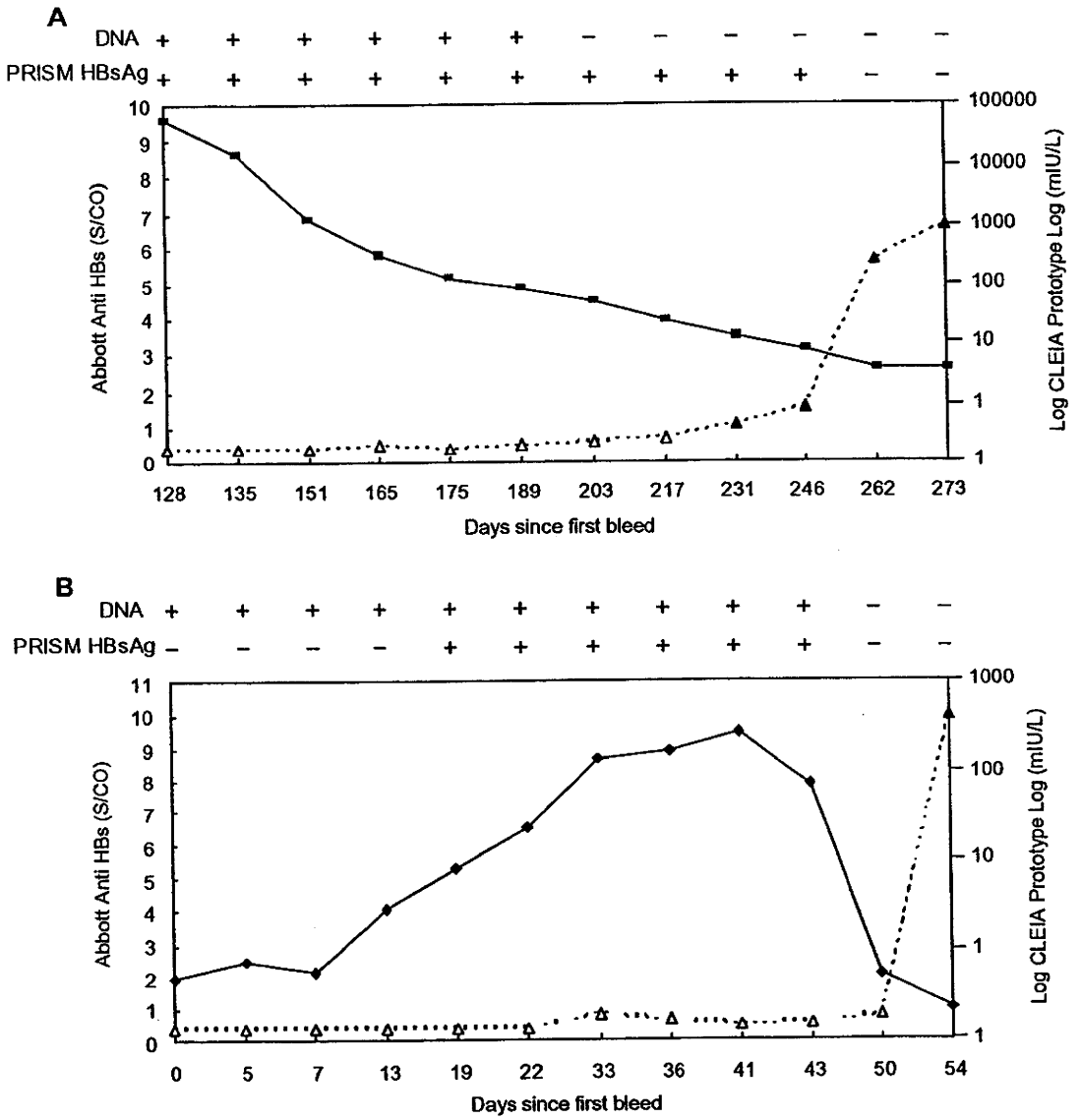

Fig. 3. The seroconversion samples that became anti-HBs–positive were measured by the CLEIA prototype. (A) PHM 935B (Boston Biomedica, Inc.). (B) HBV 6281 (Zeptometrix Corp.). The results of the CLEIA prototype (■) were compared with the DNA and PRISM HBsAg data provided (Abbott Laboratories). + = detected HBV DNA or PRISM HBsAg; − = under the detection limit for DNA or PRISM HBsAg. The appearances of anti-HBs in the serum samples were monitored using IMx AUSAB kits (Abbott Laboratories) supplied by the panel vendors. It is indicated as signal-to-cutoff ratio (S/CO). (△) Anti-HBs–negative; (▲) anti-HBs–positive. For detecting HBV DNA, the Roche Amplicor HBV Monitor assay with a detection limit of 400 copies per mL was used in PHM 935B, and the PCR conducted by the vendor with a detection limit of 100 copies/mL was used in HBV 6281.

against the WHO standard. In comparison of the cutoff point, the sensitivity of the CLEIA prototype (0.22 mIU/mL) was approximately 230-fold higher than that of the quantitative HBsAg detection system, ARCHITECT (50 mIU/mL), and 114-fold higher than that of the qualitative HBsAg detection system, PRISM (25 mIU/mL). It is suggested that the CLEIA prototype greatly improved the detection limit not only by the combination of MoAbs but also by the polymerization technique for conjugation. A dilution linearity test, which provides information about the precision of assay results for samples tested at different levels of dilution, was performed.

To evaluate whether the high-sensitivity HBsAg CLEIA prototype contributed to early detection of HBV, commercially available seroconversion panels were tested. The CLEIA prototype reduced the window period

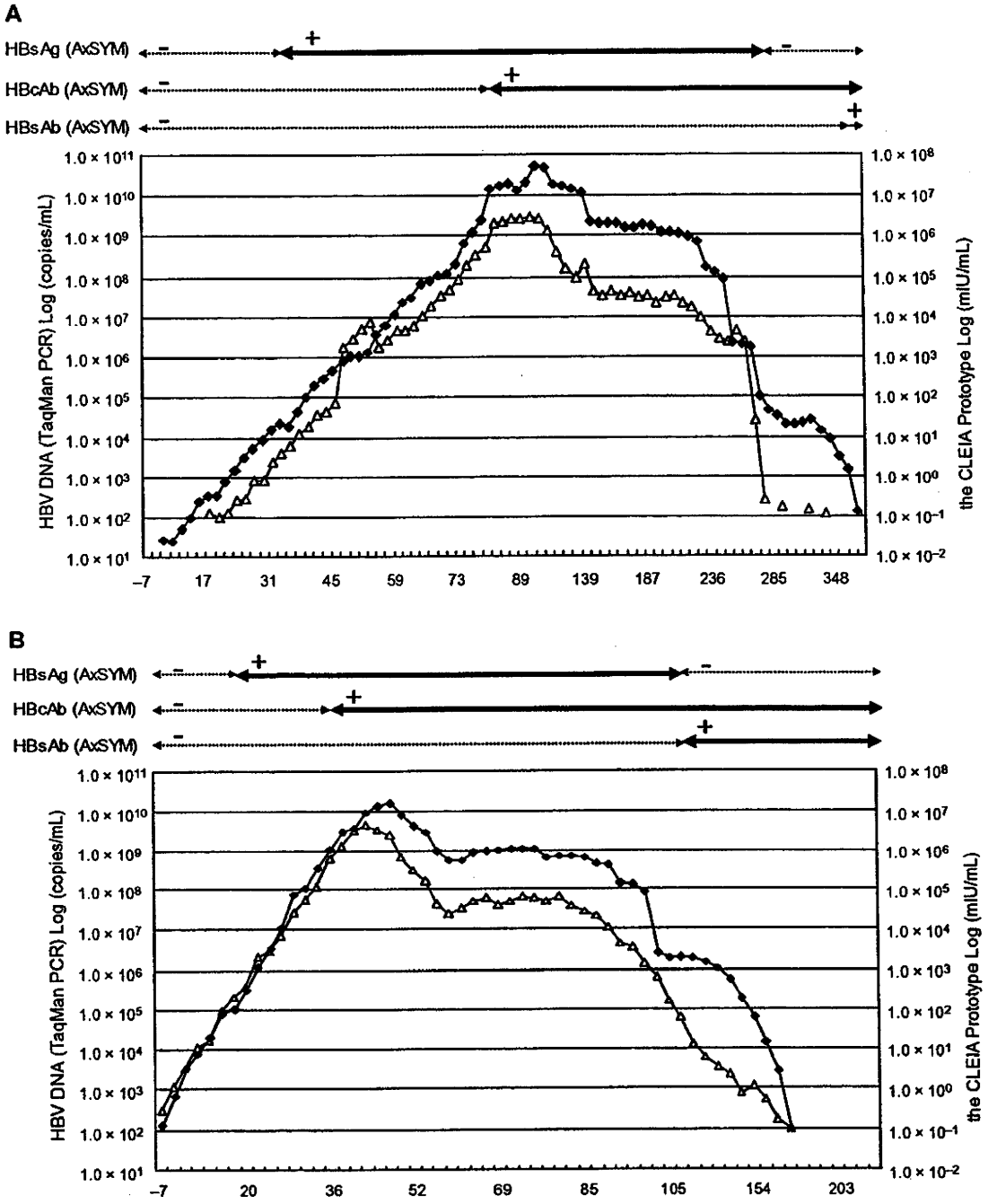

Fig. 4. The samples obtained from HBV-inoculated chimpanzees were tested by the CLEIA prototype. (A) The serum sample was collected from Chimp 246 between 1 week before inoculation and 410 days after inoculation of HBV. (B) The serum sample was collected from Chimp 272 between 1 week before inoculation and 238 days after HBV inoculation. (△) HBV DNA (TaqMan PCR); (◆) HBsAg measured by the CLEIA prototype. The data of HBsAg, HBcAb, and HBsAb measured by AxSYM (Abbott Laboratories) are shown above each graph (+ = detected; – = under the detection limit).

by 17.4 days compared with the existing CLIA; the reduced window period for HBsAg detection almost equaled that of DNA detection. In the tested 16 seroconversion panels, the results indicated that the CLEIA prototype detected HBsAg earlier than DNA in Panels PHM910 and HBV6277. However, for panel HBV6277, it was interpreted by the vendor that DNA was reactive from the first blood sample whereas it was below the level of quantitation. In addition,

TABLE 4. The first detection days of HBV DNA or HBsAg in six chimpanzees

Chimp	Genotype	Days to detection from first bleed			Windows reduction by prototype comparison with	
		DNA	AxSYM	Prototype	AxSYM	DNA
246	A	9	35	15	20	–6
280	A	55	97	69	28	–14
279	A	43	69	50	19	–7
272	C	6	22	10	12	–4
269	C	29	53	35	18	–6
285	C	29	64	50	14	–21
Mean		28.5	56.7	38.2	18.5	–9.7

since the detection limit of NAT is improved, it is considered that DNA will be detected earlier than the provided data in these two panels. This was indicated in the result of six chimpanzees compared with DNA measured by nested PCR.

The other characteristic of the CLEIA prototype was that it could detect HBsAg in the anti-HBs–positive seroconversion samples. The position of the determinant "a" is the major region detected by several commercially available HBsAg assays as well as by the naturally occurring anti-HBs after exposure to either the virus or the vaccine.[35-37] The CLEIA prototype was designed using many MoAbs for detecting various epitopes so that the HBsAg could be detected even in the anti-HBs–positive samples. It is reported that mutations occur at the many positions of "a"[17,38,39] and that individuals exposed to strains with mutations in the position of "a" may get infected despite possessing what are considered as protective titers of anti-HBs; such mutants may escape detection by some of the existing HBsAg assays.[40] Therefore, it is considered that the use of more than one MoAb to HBsAg is better.[39]

The CLEIA prototype was also assessed by measuring the serum samples derived from the chimpanzee infectious models. In the chimp infected with genotype A, HBsAg could be detected earlier than HBV DNA, both in the early and in the latter phases of infection. In contrast, in the chimp infected with genotype C, HBV DNA could be detected earlier than HBsAg both in the early phase and in the latter phases of infection. It is reported that the doubling time of genotype C was shorter than that of genotype A,[30] and the result suggested that HBsAg is affected by doubling of the HBV DNA and is correlated to the amplification. This ultrahigh-sensitive HBsAg detection is correlated to the HBV DNA, so it might be useful for monitoring patients under treatment. The utility of the CLEIA prototype for monitoring the patients in the latter phase of infection is currently being tested, and the possibility of its utility for monitor is being indicated at the moment (data not shown).

In this report, it is indicated that the sensitivity of the procedure that is commonly used for detecting HBsAg can be increased so that it reaches that of the procedure used for DNA detection. The high-sensitivity HBsAg CLEIA prototype could reduce the window period for HBV detection such that it almost equaled that of DNA detection, and it might also reduce the risk of HBV transmission caused by transfusion. Because the CLEIA prototype is not a specialized technique, it can also be performed in hospitals, and it may contribute toward reducing undesired HBV transmission. It has been reported that reactivation is induced when an individual's immune condition becomes suppressed as a result of chemotherapy, immunosuppressant treatment, or occult HBV infection.[41,42] It is suggested that the high-sensitivity HBsAg assay, which detects HBsAgs even in anti-HBs–positive samples, might be useful for monitoring chronic infection patients under treatment.

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ウイルス肝炎の臨床をめぐって

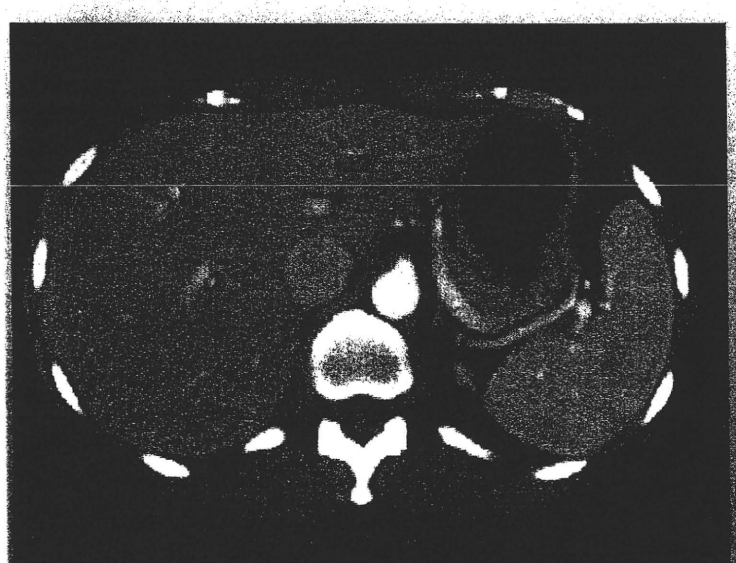
● One Point Advice

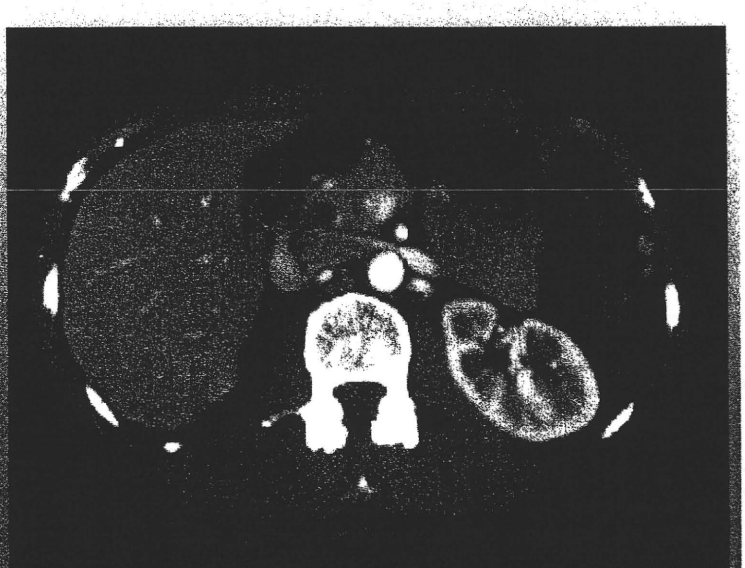
● 今月の話題
COX2阻害薬と大腸癌

● この症例から何を学ぶか
B型急性肝炎遷延化中の交際相手男性より水平感染したB型急性肝炎の1例

● 連　載
病歴と画像からすすめる神経疾患診療の実際

● 知っておきたいこと ア・ラ・カルト
加齢黄斑変性





文光堂

肝炎ウイルス検診陽性者が来院したらどうする?

—より実効性のある診療をめざして—

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はじめに●

わが国の肝炎ウイルス感染者の掘り起こしを目的として，平成14年度より老人健康法に基づく保健事業である肝炎ウイルス検診(節目検診，節目外検診)が施行され，平成18年度(今年度)で終了することになる．この5年の間に数多くの肝炎ウイルス感染者が発見されたが，その後のフォローアップ体制など種々の問題点も浮き彫りとなった．本稿では，まず肝炎ウイルス検診の概要，結果をふまえたうえで，肝炎ウイルス検診陽性者が来院したらどう対処すべきかについて考察してみたい．

肝炎ウイルス検診とは●

肝炎ウイルス検診とは，肝炎ウイルスの持続感染者(以下キャリア)の中には自覚症状がなく，感染に気づいていない者が多く，またキャリアは慢性肝炎やさらには肝硬変，肝癌へと移行する可能性があることから，自らの肝炎ウイルスの感染状況を認識する機会を供し，必要に応じて保健指導を受け医療機関を受診することにより，肝炎による健康障害を軽減し，進行を遅延させることを目的として行われるものである．

事業の実施主体は市町村であり，原則として基本健康診査時にあわせて実施されることが多い．節目検診では40歳から70歳までの5歳刻み，すなわち40・45・50・55・60・65・70歳に該当する者を対象者とし，5年間で40歳から70歳まですべての基本健康診査対象者を網羅することになる．一方，節目検診の対象とならない者のうち，過去に肝機能異常を指摘されたことのある者，広範な外科的処置を受けたことのある者または妊娠・分娩時に多量に出血したことのある者であって定期的に肝機能検査を受けていない者を対象とする節目外検診もあわせて行われている．

肝炎ウイルス検診の成果●

平成14年度から17年度までの4年間の節目検診受診率(**表1**)と肝炎ウイルス検診実績(**表2**)は表に示す通りである．これによれば過去4年間の節目検診受診率は約27%(約510万人)であった．この中でC型肝炎ウイルス(HCV)感染の可能性がきわめて高いと考えられる率が0.9%(約47,000人)，HBs抗原陽性と判定された率が1.1%(約58,000人)であった．また，節目外検診ではHCV感染の可能性がきわめて高いと考えられる者が約38,000人，HBs抗原陽性者が約25,000人であったことから，この4年間の肝炎ウイルス検診で明らかになった肝炎ウイルス陽性者は約17万人と考えられる．一方，節目検診対象者の73%(約1,300万人)は検診を受けていないことから，HCV感染の可能性がきわめて高いにもかかわらず感染を自覚していない者が約12万人(1,300万人×0.9%)，同様にB型肝炎ウイルス(HBV)感染を自覚していない者が約14万人(1,300万人×1.1%)いると推定される．節目外検診では受診対象数が同定できないため受診率を算定することはできないが，節目外検診対象者のなかにも感染を自覚していない者が相当数いると推測される．さらに，肝炎ウイルス検診対象者が国民健康保険加入者であることを考えると，それ以外の健康保険加入者は基本的に肝炎ウイルス検診を受けていないことになり，以上を総合するとまだ肝炎ウイルスに感染していながら感染を自覚していない者が数十万人は存在することを認識すべきである．

肝炎ウイルス検診陽性者への対応●

1. HCV陽性の場合

a. 診　断

肝炎ウイルス検診等実施要綱によればHCV検

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- 肝炎ウイルス節目検診受診率は約27％であった．
- 節目検診でのHCV，HBV陽性率は約1％である．
- HCVの節目外検診陽性率は節目検診陽性率の2倍強である．

表1　節目検診受診率と肝炎ウイルス検診実績　節目検診受診率(都道府県ならびに政令指定都市)

C型肝炎ウイルス

年度	受診対象者	受診者数	受診率
平成14年度	4,331,521	1,298,746	30.0%
平成15年度	4,676,854	1,375,583	29.4%
平成16年度	5,061,690	1,271,320	25.1%
平成17年度	4,848,053	1,196,457	24.7%
計	18,918,118	5,142,106	27.2%

B型肝炎ウイルス

年度	受診対象者	受診者数	受診率
平成14年度	4,331,521	1,291,195	29.8%
平成15年度	4,676,854	1,382,663	29.6%
平成16年度	5,061,690	1,279,704	25.3%
平成17年度	4,848,053	1,205,423	24.9%
計	18,918,118	5,158,985	27.3%

(厚生労働省ホームページより作成)

表2　肝炎ウイルス検診実績

C型肝炎ウイルス

年度	受診者(人)			「現在，C型肝炎ウイルスに感染している可能性がきわめて高い」と判定された者(人)			感染者率(%)		
	節目検診	節目外検診	計	節目検診	節目外検診	計	節目検診	節目外検診	全体
平成14年	1,298,746	624,734	1,923,480	14,672	16,721	31,393	1.1	2.7	1.6
平成15年	1,375,583	454,687	1,830,270	13,324	10,167	23,491	1.0	2.2	1.3
平成16年	1,271,320	347,431	1,618,751	10,385	6,446	16,831	0.8	1.9	1.0
平成17年	1,196,457	331,356	1,527,813	8,909	5,067	13,976	0.7	1.5	0.9
計	5,142,106	1,758,208	6,900,314	47,290	38,401	85,691	0.9	2.2	1.2

B型肝炎ウイルス

年度	受診者(人)			HBs抗原「陽性」と判定された者(人)			感染者率(%)		
	節目検診	節目外検診	計	節目検診	節目外検診	計	節目検診	節目外検診	全体
平成14年	1,291,195	631,918	1,923,113	15,239	9,191	24,430	1.2	1.5	1.3
平成15年	1,382,663	466,462	1,849,125	15,842	6,678	22,520	1.1	1.4	1.2
平成16年	1,279,704	356,230	1,635,934	13,950	4,804	18,754	1.1	1.3	1.1
平成17年	1,205,423	341,400	1,546,823	12,735	4,395	17,130	1.1	1.3	1.1
計	5,158,985	1,796,010	6,954,995	57,766	25,068	82,834	1.1	1.3	1.2

(厚生労働省ホームページより作成)

- 「HCV 抗体陽性」で受診したらまず HCV RNA の有無を確認する.
- HCV RNA 陰性でも肝機能異常があれば原因を精査する.
- HCV RNA 陽性が判明したら次に肝炎の有無について調べる.

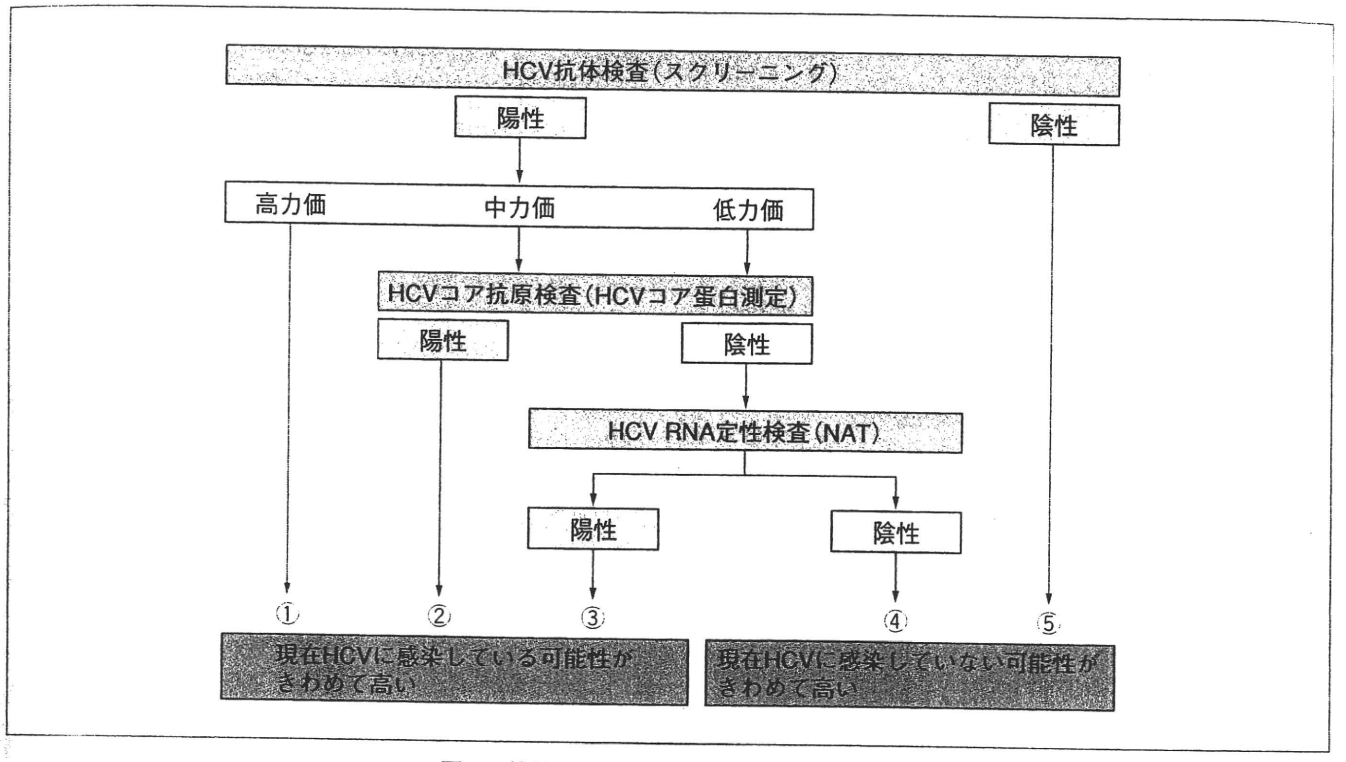

図1　検診におけるC型肝炎ウイルス検査
(文献2)より引用)

査とその判定手順は図1[2]に示す通りである．すなわち老人健康法に基づいた肝炎ウイルス検診を受けた場合はこの手順に沿って検査が行われており，いわゆるかかりつけ医などでHCV抗体検査を受けたという場合と明確に区別されなければいけない．HCV抗体検査だけの場合は低力価であれば既往感染の可能性も高いので，「HCV抗体陽性を指摘された」といって受診があった場合はまずHCV感染の有無を確認しなければならない．この場合には肝炎ウイルス検診等実施要綱と同様に，HCV抗原検査を行った後にHCV核酸増幅検査を行うか，すぐにHCV核酸増幅検査を行うかは施設により異なるのが現状である．たとえHCV感染が否定されても肝機能異常が存在すればアルコール性肝疾患や非アルコール性脂肪性肝疾患を念頭において精査を進めるべきである．これに対して肝炎ウイルス検診陽性者が受診した場合には文字通り「現在，C型肝炎ウイルスに感染している可能性がきわめて高い」と判断される．

b.　肝炎の有無について

HCV感染が確認されたら次に肝炎の有無，すなわちトランスアミナーゼが正常域か否かを判定する必要がある．近年，血清ALT値の正常値について重要な論文[1]が報告されているが，わが国においてもALT値の正常値は各施設の正常域とするのでなく，一般的に30 IU/*l*以下と考えられている．すなわちALT値が施設の正常域内であっても30 IU/*l*を超えている場合は軽度の肝炎が存在しうることを念頭におくべきである．また，たとえALT値が30 IU/*l*以下であっても1回の血液検査のみで無症候性キャリアと判断してはならない．C型慢性肝疾患の場合は肝臓の線維

肝炎の存在が疑われたら肝線維化の程度を評価する.
HCV の場合は血小板数が肝線維化の評価に有用である.
なぜ C 型肝炎に対する治療が必要であるかを自然経過と肝発癌の危険性から説明する.

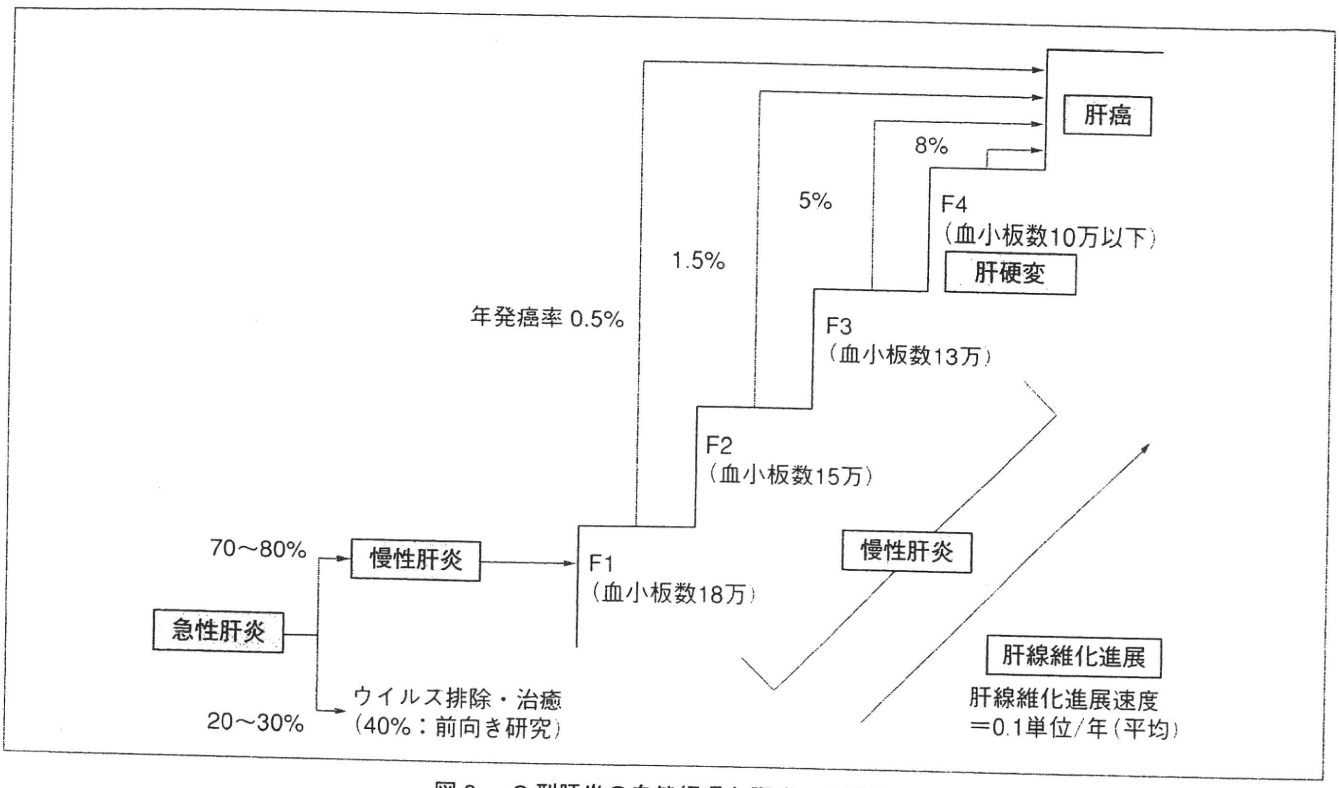

図2　C型肝炎の自然経過と肝癌への進展
(文献2)より引用)

化，すなわち staging と血小板数との相関関係が認められることから血小板数の測定も合わせて行うべきである．腹部超音波検査による肝臓の形態を把握することは肝疾患の慢性度を評価するのに重要であり，血小板数と合わせて評価することでALT 値が正常化しているような肝硬変の見落としもなくなってくる．肝生検から得られる情報は大きいが，staging に関してはある程度血小板数とも相関し，最近では欧米を中心に firoscan などの非侵襲的方法により肝線維化を評価する傾向がひろがっている．肝組織検査の適応については基礎疾患の有無，必要性など多角的に検討して決定する必要がある．

c.　治療方針の決定

肝炎の存在が考えられれば治療を行う必要がある．C 型慢性肝炎治療の第一選択はインターフェロン(IFN)(±リバビリン併用)療法であり，C 型肝炎ウイルス感染者に対する治療の標準化に関する研究班(班長：熊田博光)の治療ガイドラインによれば遺伝子型とウイルス量の違いから治療方法が異なることから，ウイルスの遺伝子型と量の測定を行うべきである．この際に重要なことはC 型慢性肝炎では自覚症状がないことがほとんどなので，なぜ治療を行う必要があるかを理解してもらうためにC 型慢性肝炎の自然経過と肝発癌の危険性(図 2)[2]について十分に説明することである．一方，先の治療ガイドラインによればALT 値正常の無症候性キャリアに対しても血小板数を考慮した治療方針が記載されているが，これについては本特集号の別項に詳細に解説されているの

HBs 抗原陽性者の場合もまず肝炎の有無を確認することが重要である.
C 型慢性肝炎にくらべて B 型慢性肝炎では血小板と肝線維化の相関は弱い.
B 型慢性肝炎では血清 ALT 値が低くても線維化が進行していることも多い.

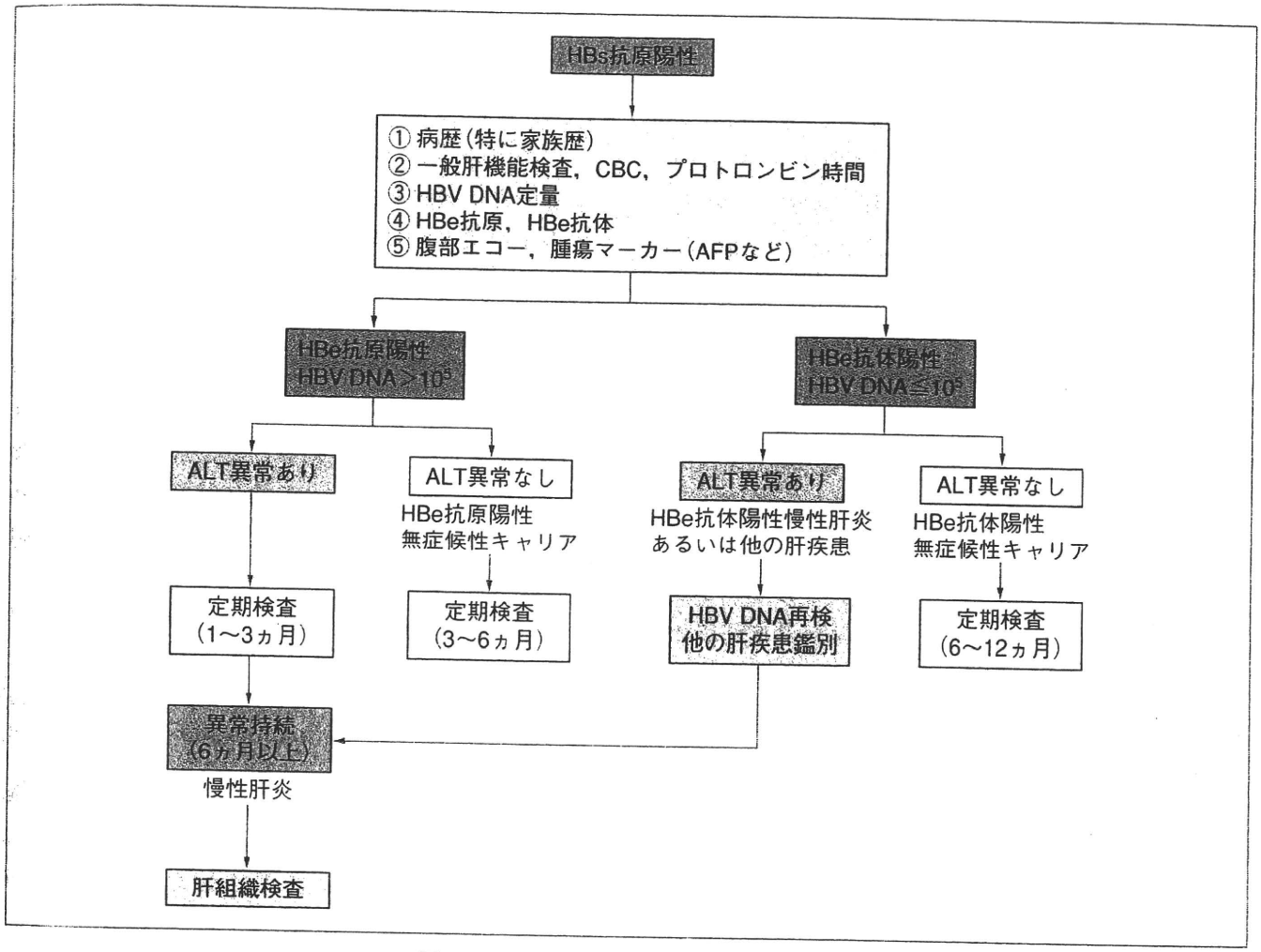

図 3　HBs 抗原陽性者に対する診断手順
(文献 2) より引用)

で省略する.

2. HBs 抗原陽性の場合

a. 診断と肝炎の有無について

急性肝炎でない限り肝炎ウイルス検診者が HBs 抗原陽性として受診した場合は HBV キャリアである. HBs 抗原陽性者に対する診断の手順については日本肝臓学会編集による「慢性肝炎の治療ガイド 2006」[2] によく書かれている(図 3). 肝炎の存在が疑われるときは, C 型慢性肝炎以上に肝組織診断(肝生検)が推奨される. これは B 型慢性肝疾患では血小板数と線維化の程度が C 型慢性肝炎ほどの相関を認めないことや, 血清 ALT 値から予測される以上に炎症や線維化が進展していることが多いためである.

b. 治療方針の決定

HBV キャリアの診療では, 患者が治療対象となるかどうかを決定することがきわめて重要である. HCV キャリアの場合は肝炎が存在すれば原則的に治療の適応があるが, HBV キャリアではたとえ HBV DNA 陽性や ALT 値の異常を認め

- 若年者のB型慢性肝炎では自然経過で肝炎が沈静化することも多い.
- B型慢性肝炎の治療適応決定のために肝生検は重要である.
- B型慢性肝炎の治療方針決定には肝臓専門医との相談も重要である.

ても，若年者ではしばしば自然経過の中でHBeセロコンバージョンし，肝炎の鎮静化を認めることを常に考慮すべきである．厚生労働省の班会議によるB型慢性肝炎の治療ガイドラインでも35歳を境に治療方針が異なっており，治療方針決定における年齢の重要性が示されている．当然のことながらHCVの場合と異なり，ウイルスの完全排除は不可能であるため，治療の目標がウイルス増殖低下に伴う肝炎の鎮静化であることをよく理解してもらう必要がある．最終的治療目標は発癌阻止，生存期間の延長，QOLの改善などである．しかし，経過中に著明なALT値の上昇を伴う急性増悪を経験することも多く，経過観察すべきか抗ウイルス療法あるいは肝庇護療法を行うべきか迷うことも少なくない．この場合に，“まだ待てる”すなわち経過観察しうるか否かの重要な判断材料は肝組織診断である．C型慢性肝炎に比べるとたとえガイドラインを参考にしても治療方針に苦慮することも多く，症例に応じた個々の対応を求められることが多いのがB型慢性肝炎の特徴でもあり，肝臓専門医に相談することも重要な手段であることを認識していただきたい．

おわりに

この4年間の肝炎ウイルス検診により新たな肝炎ウイルスキャリアが発見されたが，肝炎ウイルス感染者に対して適切な対応をとってこそ検診の価値が発揮されることになる．肝炎ウイルス検診陽性者が受診した際には，肝炎が存在するか否か，治療が必要か否かについて判断し説明を行うことが重要である．また，たとえ肝炎が存在しなくてもウイルス性肝炎の自然経過を説明することにより定期的な経過観察が必要であることを理解してもらうように努めるべきである．

文　献

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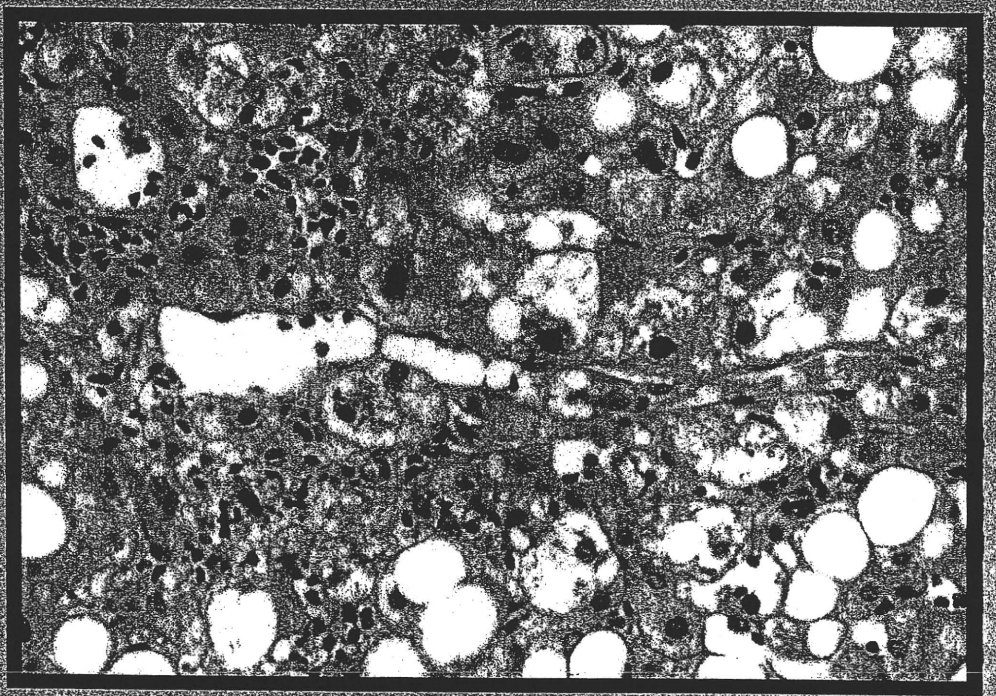

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