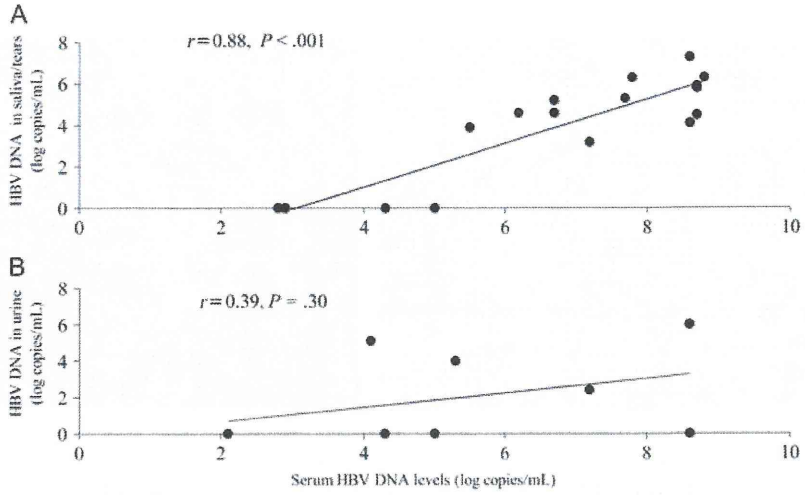

Figure 4. *A*, The association between hepatitis B virus (HBV) DNA levels in serum samples and saliva and tear samples (n = 18). Data from patients whose levels of HBV DNA in serum ranged from 2.9 to 8.8 log copies/mL were used for analysis. There was a significant correlation between HBV DNA levels in serum specimens and saliva and tear specimens ($r = 0.88$; $P < .001$). *B*, The association between HBV DNA levels in serum and urine samples (n = 9). Data from patients whose serum HBV DNA levels ranged from 2.1 to 9.0 log copies/mL were used for analysis. There was no significant correlation between HBV DNA levels in serum and urine specimens ($r = 0.41$; $P = .10$).

association between HBV DNA loads in serum and urine specimens (HBV DNA levels in 9 serum specimens ranged from 2.1 to 8.6 log copies/mL; $r = 0.39$; $P = .30$) (Figure 4*B*).

Transmission of HBV by Tears

The level of HBV DNA in tear specimens collected from a 10-month-old girl (genotype C; serum HBV DNA load, >9.0 log copies/mL) were 7.1 log copies/mL. The final concentration of HBV DNA in filter-sterilized tear specimens was 6.1 copies/mL.

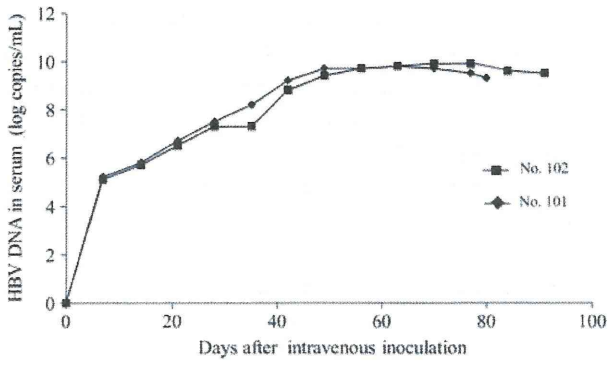

Figure 5. Hepatitis B virus (HBV) DNA levels in serum from chimeric mice after intravenous inoculation with tear specimens. The level of HBV DNA in a tear specimen collected from a girl with failure of immunoprophylaxis (HBV DNA load in serum, >9.0 log copies/mL) was 7.1 log copies/mL. After sterilization, the final concentration of HBV DNA in the tear sample was 6.1 copies/mL. One hundred microliters of the tear specimen was injected intravenously into chimeric mice.

A total of 100 μL of the filter-sterilized tear specimen was injected intravenously into 2 chimeric mice. One week after inoculation, both chimeric mice became positive for HBV DNA in serum (no. 101 had an HBV DNA level of 5.2 log copies/mL, and no. 102 had an HBV DNA level of 5.1 log copies/mL). The levels of HBV DNA in serum from the chimeric mice gradually increased with time. Seven weeks after inoculation, the levels of HBV DNA in serum from the chimeric mice increased to 9 log copies/mL and remained at this level thereafter (Figure 5). Saliva and lacrimal fluids were collected using FTA cards at day 80 (for mouse 101) and day 91 (for mouse 102). Although HBV DNA was extracted from a very small spot (1 pinched-out circle from the FTA card), the levels of HBV DNA were 4.4 log copies/mL (in saliva) and 4.5 copies/mL (in lacrimal fluids) in mouse 101 and 4.0 log copies/mL (in saliva) and 4.3 log copies/mL (in lacrimal fluids) in mouse 102. The remaining chimeric mouse (mouse 103) was orally inoculated with 100 μL of the filter-sterilized tear specimen. Unfortunately, we had to discontinue oral administration because of the deterioration of the mouse's health 35 days after inoculation. The chimeric mouse (mouse 103) had been inoculated orally twice (on days 0 and 28) before discontinuation. Real-time PCR performed 6 times (on days 0, 7, 14, 21, 28, and 35) detected no HBV DNA in serum.

Immunohistological Analysis of Liver Tissue for HBV Antigens

Immunohistochemical staining was performed on a liver specimen from the mouse with HBV viremia (no. 101). The hepatocytes were positive for HBsAg and HBcAg (Figure 6).

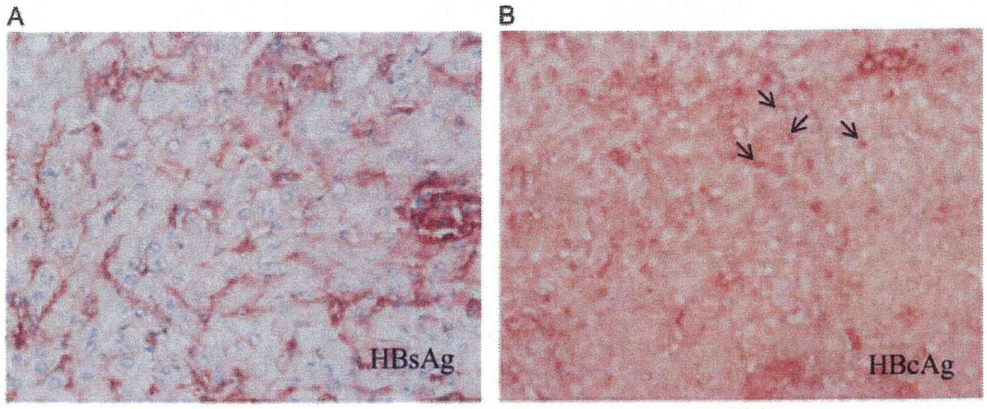

Figure 6. Immunohistological staining for liver tissue with antibodies to hepatitis B virus (HBV) surface antigen (HBsAg) and HBV core antigen (HBcAg). *A*, HBsAg was expressed on cytoplasmic membrane (original magnification ×400). *B*, HBcAg were expressed in nuclei of hepatocytes (original magnification ×400). Arrows indicate the nuclei of HBcAg-positive staining.

These findings indicated that HBV transmission from tears could be replicated in a human liver chimeric mouse model.

DISCUSSION

Although it has been reported that HBV DNA was detectable by PCR in tears from chronic HBV carriers [11, 17], tears have been considered to be low risk for HBV transmission. However, this study demonstrated that tears from children chronically infected with HBV were highly infectious. HBV DNA from serum could be detected in both chimeric mice 1 week after inoculation. Moreover, the levels of HBV DNA in serum continuously increased and reached the upper limit of the PCR assay 7 weeks after inoculation. A previous study showed that chimeric mice usually became positive for HBV DNA in serum 4 weeks after intravenous inoculation with serum from HBV carriers [28]. The levels of HBV DNA in tears used for this study were much higher than those in serum used in the previous study. Therefore, HBV DNA in serum from the chimeric mice became detectable quickly after inoculation.

Recent studies measuring HBV DNA in body fluids from HBV carriers have been conducted in the Netherlands, Sweden, and Denmark. Including the present study, all studies are from counties in which a selective HBV immunization program has been implemented [9–13]. Clearly, physicians from these countries are keen to know whether various body fluids might be sources of HBV transmission. Additionally, physicians are concerned that a vaccination strategy that focuses on at-risk groups is ineffective for prevention of HBV infection. Although recent studies have shown that HBV DNA in urine, saliva, tear, and sweat specimens from chronic HBV carriers was detectable by PCR, these studies did not show that body fluids from chronic HBV carriers were infectious in animal experiments. Approximately 30 years ago, the infectivity of semen and saliva from HBV carriers was proven by experimental transmission, using gibbons [20, 21]. Since then, no other body fluids have been evaluated for infectivity. This study is the first to confirm that tears are infectious sources of HBV.

Tears are presumed to originate from circulating blood. HBV DNA was first detected in tears in 1994 by PCR. In a previous study, tear specimens from 47.1% of HBV carriers (16 of 34) were positive for HBV DNA [17]. In 2006, a previous study measured HBV DNA in paired saliva and tear specimens. Of 7 patients with chronic HBV infection, 4 (57%) had tear specimens that were positive for HBV DNA. The levels of HBV DNA in tear specimens ranged from 0.2×10^3 to 1.4×10^4 copies/mL [11]. Compared with the previous study, the levels of HBV DNA in tears were relatively high in this study. There are 2 possible explanations for the difference in HBV DNA levels between these studies. First, the majority of the patients supplying tear samples in our study were very young children (median age, 1 year). Young children with chronic HBV infection are usually in the immunotolerant phase and have a high viral load. Second, the FTA card was effective at collecting body fluids and extracting DNA. Although the number of tear samples was small, this study demonstrates that tears, as well as saliva, contain a large amount of HBV DNA. Interestingly, HBV DNA in lacrimal fluid and saliva could also be detected in the chimeric mice. These findings suggest that tears, like saliva, have the potential to transmit HBV.

Among body fluids, the highest levels of HBV DNA are detected in blood. However, HBV DNA can also be detected in urine, saliva, tears, and sweat. In this study, HBV DNA was detected in a high proportion of body fluid samples. In addition, there was a statistically significant correlation in the

levels of HBV DNA between tear and saliva specimens and serum specimens, in which [log HBV DNA level in saliva and tear specimens] = $-3.23 + 1.06 \times$ [log HBV DNA level in serum specimens]. Similarly, previous studies reported that the levels of HBV DNA in saliva specimens were significantly related to the levels of HBV DNA in blood specimens. In this study, however, the levels of HBV DNA in urine specimens were not significantly associated with the levels of HBV DNA in serum specimens. The levels of HBV DNA in urine samples were significantly lower than those in saliva and tear samples. This finding is also consistent with that of a previous study [13]. We cannot provide any clear explanation why the levels of HBV DNA were lower than those in other body fluids. Further studies are required to study not only the infectivity of urine but also the mechanism of the reduction of the HBV DNA level in urine.

It has been known that the oral administration of serum from HBV carriers causes HBV infection [19]. After we confirmed the infectivity of tears through the intravenous route, tears were administered orally to a chimeric mouse. Although both transmission routes were investigated using the same sample, this study, like previous animal experiments [20, 21], failed to demonstrate that HBV infection occurred through an oral route; unfortunately, the period of observation was not sufficient to evaluate the infectivity of tears. We tried to detect HBV DNA in the liver of chimeric mouse 103 after discontinuation of oral administration of tear specimens, but HBV DNA was not detectable in the liver by real-time PCR (data not shown).

There are few studies that have measured the levels of HBV DNA in sweat specimens from chronically infected patients. A previous study quantified HBV DNA levels in Olympic wrestlers, who were negative for HBsAg but positive for HBV DNA in blood [14]. In the previous study, a statistically significant relation between the levels of HBV DNA in blood and sweat was observed. In the present study, all sweat samples were positive for HBV DNA. In addition, the levels of HBV DNA in sweat specimens were high (mean level [±SD], 5.2 ± 0.6 log copies/mL). Therefore, sweat from HBV carriers might also have the potential to cause horizontal HBV infection.

The US Centers for Disease Control and Prevention considers that the risk of transmission in child-care settings is very low [29–31]. However, Ireland, Norway, and Sweden have a policy that children should be immunized if another child in a day care center is positive for HBsAg. This study showed that various body fluids from young HBV carriers have a high concentration of HBV DNA. Previous studies have reported that 10% of HBV particles are infectious [32]. Therefore, all body fluids from HBV carriers should be considered to be infectious, and HBV vaccine should be recommended for day care staff.

In conclusion, HBV DNA was detected at high proportions in urine, saliva, tear, and sweat specimens from chronic HBV carriers. The levels of HBV DNA in saliva and tear specimens from young children were extremely high. In addition, tear samples from a child with chronic HBV infection were confirmed to be infectious, using chimeric mice. Although the HBV transmission risk between young children in nurseries or day care centers may be limited, strict precautions should be taken against contact with body fluids from HBV carriers with high-level viremia, especially in counties implementing an immunizing program focused on individuals at-risk for HBV infection.

Notes

Financial support. This work was supported by the Ministry of Health, Labor, and Welfare of Japan.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Hepatology Research

Hepatology Research 2011; 41: 399–404 doi: 10.1111/j.1872-034X.2011.00789.x

Original Article

Efficacy of pegylated interferon-α2a monotherapy in Japanese children with chronic hepatitis C

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Aim: There is little information available on the efficacy of pegylated interferon (PEG IFN) therapy for children with chronic hepatitis C. The aim of this study was to evaluate the efficacy and tolerability of PEG IFN-α2a monotherapy for children infected by chronic hepatitis C virus (HCV).

Methods: From 2004–2006, we conducted a prospective, open-label, multicenter study of 22 patients aged 4–18 years, including eight with genotype 1 and 14 with genotype 2. None had previously received IFN. The patients were treated with s.c. PEG IFN-α2a at a dose of 3 μg/kg once a week for 48 weeks. Rapid virological response (RVR) was defined as: undetectable serum HCV RNA at week 4; early viral response (EVR) as a 2 or more log reduction or undetectable serum HCV RNA at week 12; and sustained viral response (SVR) as undetectable serum HCV RNA at 24 weeks after the cessation of treatment.

Results: SVR was achieved in 10 (45%) of the 22 patients (three with genotype 1, seven with genotype 2). Retrospectively, the patients with SVR included five with RVR (one with genotype 1, four with genotype 2) and five with EVR (two with genotype 1, three with genotype 2). PEG IFN-α2a monotherapy was well tolerated, except in one patient in whom alanine aminotransferase activity flared (>500 IU/L) during treatment.

Conclusion: The efficacy of PEG IFN-α2a monotherapy in children is similar to that for adults, while tolerability seems to be better in children than in adults.

Key words: hepatitis C virus, monotherapy, pediatric, pegylated interferon-α2a

INTRODUCTION

THE ESTIMATED WORLDWIDE prevalence of hepatitis C virus (HCV) infection is 3%, with 170 million people affected by the virus.[1] Chronic hepatitis C is a major cause of cirrhosis and hepatocellular carcinoma. The current standard therapy for chronic hepatitis C in adults is a combination of pegylated interferon (PEG IFN) and ribavirin given for 24 or 48 weeks, depending on the viral genotype.[2–5] However, there are only a few reports on therapy for children with chronic hepatitis C,[6–10] and only Schwarz *et al.*[10] have examined the efficacy of PEG IFN-α2a monotherapy in children with chronic hepatitis C. There is also a concern about possible teratogenic adverse effects of ribavirin. Therefore, we conducted an open-label, multicenter study to evaluate the efficacy, safety and tolerability of PEG IFN-α2a monotherapy in HCV-infected Japanese children.

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Received 20 October 2010; revision 11 January 2011; accepted 23 January 2011.

METHODS

Study design

PEGYLATED INTERFERON-α2A monotherapy was performed in 22 children who were enrolled by the Japan Society of Pediatric Hepatology (JSPH) from

January 2004 to December 2006. The institutional review boards of the participating centers approved the protocol, and we obtained written informed consent from parents of all the patients. HCV genotyping was assayed by sequence analysis of a portion of the 5′-untranslated region of the HCV genome before treatment was initiated.

Patients

The 22 patients were aged from 4–18 years and all had chronic hepatitis C diagnosed based on detectable serum HCV RNA titers (>50 IU/mL) for 3 years or more with continuously or intermittently elevated alanine aminotransferase (ALT) values. Serum HCV RNA titers were required to be detectable for a minimum of 3 years because spontaneous HCV resolution occasionally occurs in children less than 3 years old after exposure to HCV.

Patients were excluded from the study if they had neutropenia ($<1.5 \times 10^9$/L), anemia (hemoglobin <10 g/dL), thrombocytopenia ($<90 \times 10^9$ cells/L) or decompensated liver disease. Those positive for HIV and those with non-HCV liver disease were also excluded. The exclusion criteria also included coexisting serious medical or psychiatric illness. All children were negative for autoimmunity markers: antinuclear antibody (ANA), smooth muscle antibody, and liver-kidney microsomal antibody type 1 (LKM1). A needle liver biopsy was performed before treatment and histological evaluation was conducted using METAVIR scores in order to determine chronic hepatitis which should deserve antiviral treatment even in patients with normal transaminase levels.[11] Liver biopsy was performed under intravenous anesthesia.

Treatment

The dose of PEG IFN-α2a was based on the adult dose (180 μg per injection), modified to account for the smaller size of the children. PEG IFN-α2a was given as a once-weekly s.c. injection of 3 μg/kg for 48 weeks. Treatment was discontinued at weeks 4 or 12 based on the judgment of each pediatrician, if the serum HCV RNA did not show qualitative (HCV RNA negative) or quantitative (>2 $\log_{10}$ decrease in HCV RNA from baseline) changes, or if other serious adverse effects occurred due to PEG IFN-α2a. Patients who completed the treatment were followed for at least 24 weeks after the end of therapy.

Assessment of efficacy and safety

Hepatitis C virus RNA titers were measured using a polymerase chain reaction (PCR) assay (Cobas Amplicore HCV Monitor ver. 2.0; Roche Diagnostic Systems, Tokyo, Japan) at weeks 4, 12, 24 and 48, and at week 72 of follow up (week 72 was defined as 24 weeks after cessation of treatment). The range of the assay was 5.0×10^3 to 5.0×10^6 IU/mL. When the measured serum HCV RNA level was 5.0×10^3 IU/mL, HCV RNA was also determined by a quantitative PCR assay (Amplicor HCV ver. 2.0; Roche Diagnostic Systems), which had a detection limit of 50 IU/mL. A rapid virological response (RVR) was defined as undetectable serum HCV RNA at week 4 of treatment, using a quantitative PCR assay. An early virological response (EVR) was defined as a 2 or more log reduction (partial EVR) or complete absence of serum HCV RNA (complete EVR) at week 12 of therapy compared with the baseline level. The primary efficacy outcome was the incidence of sustained virological response (SVR), which was defined as an undetectable serum HCV RNA at 24 weeks after cessation of treatment.

The PEG IFN-α2a dose was transiently reduced by half in patients with a neutrophil count of less than 0.75×10^9/L or a platelet count of less than 50×10^9/L. PEG IFN-α2a was temporarily discontinued if the neutrophil count fell to less than 0.5×10^9/L or the platelet count fell to less than 25×10^9/L. PEG IFN-α2a was resumed once the neutrophil count exceeded 0.5×10^9/L and the platelet count exceeded 25×10^9/L.

Statistical analysis

Analysis of the relationship between baseline characteristics and response was performed by Fisher's exact test. Confidence intervals were set at 95% and a $P \leq 0.05$ was considered significant.

RESULTS

Baseline characteristics

THE BASELINE CHARACTERISTICS of the 22 patients are shown in Table 1. Eight patients had genotype 1b and 14 had genotype 2a or 2b. Five patients (23%) had a significant disease history, including acute myeloid leukemia ($n = 1$), hereditary spherocytosis ($n = 1$), congenital heart disease ($n = 1$), Down syndrome ($n = 1$) and membranoproliferative glomerulonephritis ($n = 1$). These diseases were in remission or stable at the time of PEG IFN therapy, except membranoproliferative glomerulonephritis. This case was successfully treated with PEG IFN therapy and her renal condition was improved.[12] All patients were naïve cases with respect to IFN therapy. Quantitative tests indicated

Table 1 Baseline characteristics of patients ($n = 22$)

Characteristics	Value
Age (range), year	12 (4–18)
ALT (range), IU/L	48 (15–169)
HCV RNA (range), IU/mL, log 10	5 (3–6)
Genotypes, *n* (%)	
1b	8 (36)
2a	9 (41)
2b	5 (23)
Underlying diseases, *n* (%)	5 (23)
Route of infection, *n* (%)	
Mother-to-infant	14 (64)
Blood-borne	6 (27)
Unknown	2 (9)
Treatment naïve, *n* (%)	22 (100)

ALT, alanine transaminase; HCV, hepatitis C virus.

a viral load of more than 10^5 IU/mL in 18 patients (82%). None of the patients had symptoms consistent with liver disease. Liver biopsies were performed in 21 patients (the parents of the patient with Down syndrome did not provide informed consent to this procedure): 18 (86%) had grade A1 and three (14%) had grade A2; and eight (38%) had stage F0; 10 (48%) had stage F1; and three (14%) had stage F2.

Overall efficacy

Sustained virological response was achieved in 10 (45%) of 22 patients: in three (38%) of eight with genotype 1 and in seven (50%) of 14 with genotype 2 (Fig. 1). Fifteen patients (68%) had abnormal ALT values during the therapy. There was no significant relationship of SVR with genotype 1 or 2 (P = NS). In one patient with genotype 2, ALT levels exceeded 500 IU/L

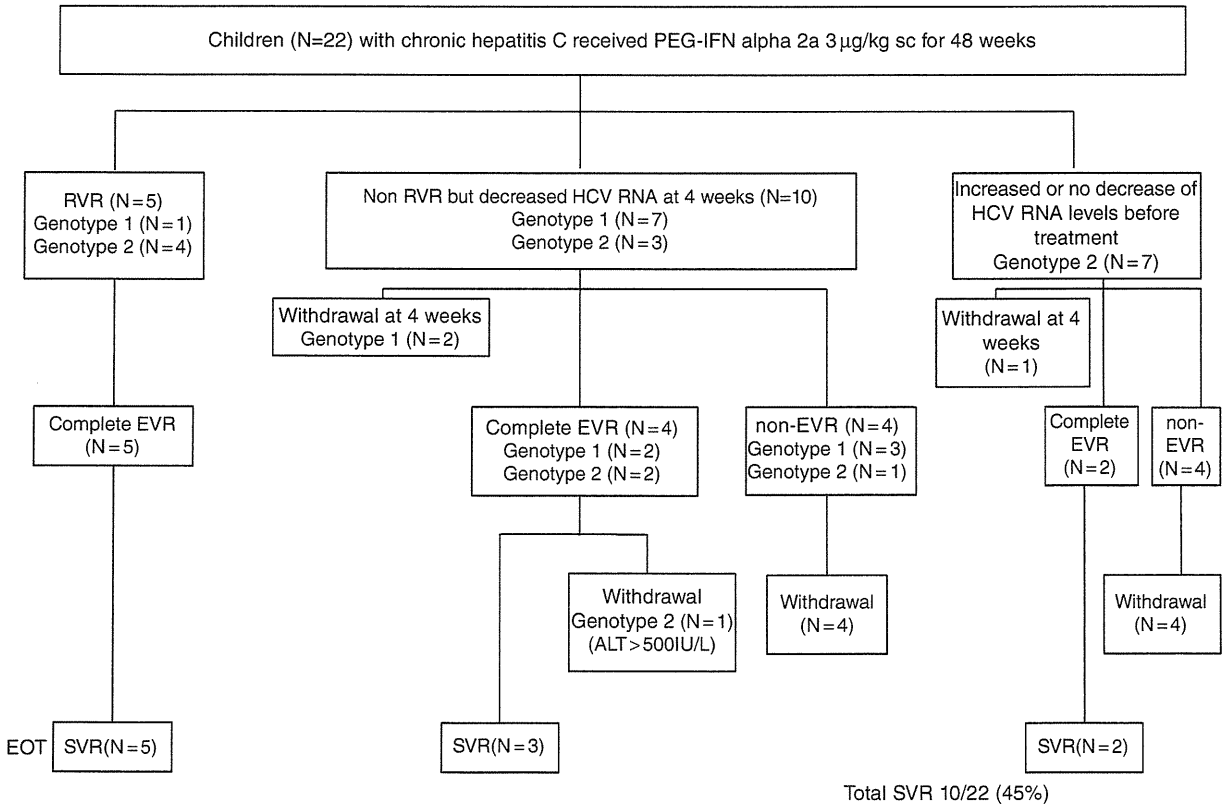

Figure 1 A total of 22 children (eight with genotype 1, 14 with genotype 2) were enrolled in the study. RVR and EVR occurred in five and 11 patients, respectively. SVR was achieved in 10 (45%) (three with genotype 1, seven with genotype 2). All those who achieved RVR also accomplished SVR. Therapy was discontinued in 12 patients (three without RVR, eight without EVR, one with ALT flare up). ALT, alanine transaminase; EVR, early virological response; HCV, hepatitis C virus; PEG IFN, pegylated interferon; RVR, rapid virological response; SVR, sustained virological response.

Table 2 Adverse events during therapy

Events	*n* (%)
Influenza-like symptoms	15 (68)
Increased ALT levels	15 (68)
Injection site reaction	7 (32)
Gastrointestinal symptoms	6 (27)
Mild alopecia	6 (27)
Skin rash	3 (14)
Deterioration of allergic diseases	2 (9)
Stomatitis	2 (9)
Retinopathy	1 (5)

ALT, alanine transaminase.

and the therapy was stopped at week 12. The ALT titers returned to normal after cessation of therapy and SVR was achieved.[12]

Virological response

Early viral response is the most accurate predictor of SVR in adult series.[13,14] Therefore, the eight children (three genotype 1, five genotype 2) who did not achieve EVR in our series discontinued therapy at week 12. RVR is also highly predictive of SVR independently of genotypes and regardless of the treatment regimen.[15] Thus, three patients who did not achieve RVR discontinued therapy at week 4. The other 10 patients completed the therapy and attained SVR. All five patients (one genotype 1, four genotype 2) who achieved RVR attained SVR. The other six patients (two genotype 1, four genotype 2) who did not achieve RVR attained complete EVR, even though HCV RNA levels were unchanged in two of these six patients at week 4. Complete EVR was achieved in 11 (58%) of 19 patients: in all three (100%) with a low viral load ($\leq 1.0 \times 10^5$ IU/mL) and in eight (50%) of 16 with a high viral load ($>1.0 \times 10^5$ IU/mL). There was no significant relationship of SVR with complete EVR or viral load (P = NS).

Adverse effects

Discontinuations and adverse events are shown in Table 2. Twelve patients (55%) discontinued the therapy because of a lack of response to therapy (three at week 4; eight at week 12) or highly increased ALT levels (one at week 12). Patients who discontinued therapy because of abnormal ALT levels (>500 IU/l) attained SVR, as previously described.[12] The main adverse events related to the therapy were flu-like symptoms including fever, lethargy and joint pain (n = 15, 68%). Abnormal serum ALT levels continued during treatment in 15 of 22 patients (68%), but improved after the therapy. No patients required dose reduction of PEG IFN-α2a due to leucopenia, anemia or thrombocytopenia; and none withdrew because of adverse events.

DISCUSSION

THE EMERGENCE OF PEG IFN-α has released patients from the burden of frequent injections, and a higher rate of SVR can be achieved compared to conventional IFN treatment in adults.[16] A combination of PEG IFN-α plus ribavirin is the current standard therapy for chronic hepatitis C in adults, and the efficacy of this therapy has been confirmed in many studies.[13,17,18] There are a few reports available on the efficacy of PEG IFN therapy for children with chronic hepatitis C including only one report of Japanese children with chronic hepatitis C.[19] The timing of starting the treatment seems to be controversial, because chronic hepatitis C in children tends to have a much more benign course than adults.[20] But the quality of life (QOL) is reduced in children with chronic hepatitis C with respect to both mental and physical meanings, so early treatment is one of the recommendable choices.[21,22] In Japan, IFN therapy should be recommended 3 years after HCV exposure. Conventional IFN monotherapy in children gives an SVR rate of 33–45% (26% in genotype 1, 70% in other genotypes), which is much better than the rate of 19% observed in adults.[23–25] The better response rate in children may be the result of the short time between HCV infection and treatment, the relatively higher dose of IFN and lack of comorbid diseases.[10,26]

An SVR rate of approximately 30% is obtained with PEG IFN monotherapy in adults.[13,23] The exact SVR rate of PEG IFN monotherapy in children is uncertain because only a small number of pediatric cases have been published. Our study showed an overall SVR rate of 45%, which is similar to the 43% rate found in one previous report.[10] Three of eight patients with genotype 1 achieved SVR, and this rate was not so high compared to conventional IFN monotherapy for 6 months, as we previously described (43% vs 40%).[25] A recently published abstract from the Peds-C trial showed an SVR rate with PEG IFN monotherapy of only 21%.[27] Therefore, SVR rates in children show little or no difference between conventional IFN monotherapy and PEG IFN monotherapy, whereas a significant difference is observed in adult cases.[27]

A weekly injection without daily medication is advantageous for the QOL of pediatric patients. PEG IFN-α2a is produced by covalent attachment of recombinant

IFN-α2a to a branched mobile 40-kDa polyethylene glycol moiety, which shields IFN-α2a from enzymatic degradation, reduces systemic clearance and enables once-weekly administration. Schwarz *et al.*[10] studied the pharmacokinetics of PEG IFN-α2a in 14 children aged 2–8 years. The dose was calculated using the formula: body surface area (m^2)/(1.73 m^2) × 180 μg, and was s.c. injected once a week for 48 weeks. At week 24, the mean trough concentration was approximately 20% less than that in adults. The area under the concentration-time curve during the 0–168-h time interval at week 24 was 25% higher than that in adults.[10]

As mentioned above, PEG IFN treatment gives higher SVR rates than conventional IFN treatment in adults, but in children the SVR rates seem to be lower in PEG IFN-α2a monotherapy than in conventional IFN therapy. Exposure to PEG IFN is higher in children compared to adults, but the SVR rate is relatively lower than in adults. This suggests that the clearance of PEG IFN in children may be higher than in adults. Regarding safety, PEG IFN was generally well tolerated in our series. Transient flu-like symptoms were the major adverse events, but dose reduction was not required. Many previous studies have also reported good tolerance of antiviral drugs for chronic hepatitis C in pediatric patients.[7,24,26]

In conclusion, the effectiveness of conventional IFN monotherapy is higher than that of PEG IFN monotherapy in children, which suggests that clearance of PEG IFN in children may be faster than that in adults. Therefore, high-dose PEG IFN therapy or a shortened interval between PEG IFN injections should be considered to achieve further improvement of the SVR rate in pediatric patients.

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J Infect Chemother (2011) 17:807–811
DOI 10.1007/s10156-011-0267-5

ORIGINAL ARTICLE

Evaluation of a simultaneous detection kit for the glutamate dehydrogenase antigen and toxin A/B in feces for diagnosis of *Clostridium difficile* infection

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Received: 10 February 2011 / Accepted: 30 May 2011 / Published online: 2 July 2011

Abstract Rapid detection kits for toxin A/B in feces are widely used as a diagnostic tool for *Clostridium difficile* infection (CDI). Their low sensitivity, however, has been considered a problem. In this study, we evaluated a new rapid diagnostic kit for simultaneous detection of the glutamate dehydrogenase (GDH) antigen and toxin A/B, *C. DIFF* QUIK CHEK COMPLETE. A total of 60 stool specimens from 60 patients with antibiotic-associated diarrhea were examined. Using *C. difficile* culture as the reference method, the GDH portion of this kit indicated a sensitivity, specificity, and negative predictive value of 100, 93.3, and 100%, respectively. The toxin A/B portion showed a sensitivity and specificity of 78.6 and 96.9%, respectively, compared to the culture results of toxin B-positive *C. difficile* (toxigenic culture). Of the 23 specimens that showed "dual positives" for GDH and toxin A/B, 22 were toxigenic culture positive, whereas *C. difficile* culture was negative in all the 28 specimens that showed "dual negatives" for GDH and toxin A/B. Of the nine "GDH-positive and toxin A/B-negative" specimens, six exhibited positive results by toxigenic culture. Results showing "dual positives" and "dual negatives" for GDH and toxin A/B can be reported as "true positive" and "true negative," respectively, whereas additional testing for confirmation, such as toxigenic culture, is required for specimens with discrepant results. Diagnostic algorithms, utilizing the simultaneous detection kit for GDH and toxin A/B as an initial screening test, may be useful for accurate and efficient diagnosis of CDI as well as the control of healthcare-associated infections.

Keywords *Clostridium difficile* infection · Glutamate dehydrogenase · Toxin A · Toxin B · Laboratory diagnosis · *Clostridium difficile* culture

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Introduction

Clostridium difficile is a major causative agent of antibiotic-associated diarrhea and colitis. It can lead to a variety of clinical manifestations ranging from mild diarrhea to severe forms of intestinal illness including pseudomembranous colitis, ileus, toxic megacolon, and bowel perforation. *C. difficile* is a spore-bearing obligate anaerobe and is resistant to oxygen or antiseptics, depending on its spore status, thus making it possible for the pathogen to reside in the hospital environment for long periods and ultimately to cause healthcare-associated infections [1–4].

Toxins A and B have been well documented as major virulence factors of *C. difficile*. Toxin A-positive, toxin B-positive (A^+B^+) strains as well as toxin A-negative, toxin B-positive (A^-B^+) strains are known to cause diarrhea and colitis, and detection of toxin B in feces is important for the diagnosis of *C. difficile* infection (CDI). The traditional gold standard is a cytotoxin assay that detects the cytotoxicity of

toxin B in fecal eluate by using cell culture, but this assay requires special equipment and techniques, making it unfeasible in many clinical laboratories [3, 4]. Rapid detection tests for toxin A/B by enzyme immunoassay (EIA) are widely used because of their ease of use and lower labor costs, but these tests appear to have a low sensitivity, which leads to concern about false-negative results [5–7]. Polymerase chain reaction (PCR) tests that detect the toxin B gene in stool samples may be rapid and more sensitive than EIA-based toxin detection, but it would be difficult for many ordinary clinical laboratories to employ these as routine diagnostic tests because acquisition of the instrument requires an initial large expenditure [7]. *C. difficile* culture followed by detection of a toxin-producing isolate (toxigenic culture) is considered the most sensitive diagnostic method. However, the turnaround time of at least 2–3 days until reporting of the final results is too long, and performing toxigenic culture on all the stool specimens submitted for *C. difficile* testing is labor intensive [8, 9].

Rapid detection tests for glutamate dehydrogenase (GDH), a cell-wall protein of *C. difficile*, have also been utilized as a diagnostic tool for CDI, although they provide no information about the toxigenicity. In contrast to the GDH detection tests by latex agglutination assay, those by EIA show a high sensitivity and negative predictive value, making them useful for rapid screening of *C. difficile* strains in feces. To make an accurate and efficient diagnosis of CDI, researchers have proposed testing algorithms, such as one that consists of an initial screening by EIA-based GDH detection, a rapid toxin detection test for GDH-positive specimens, and a final confirmatory test for GDH-positive/toxin-negative specimens by a cytotoxin assay, toxigenic culture, or PCR [3, 4, 8, 9].

Recently, an EIA-based rapid combination test kit for GDH and toxin A/B in feces, the *C. DIFF* QUIK CHEK COMPLETE (TECHLAB, Blacksburg, VA, USA) (*C. DIFF* COMPLETE), has become available in countries outside Japan and appears to be a promising aid in improving the accuracy and efficiency of CDI diagnosis [10–12]. In this study, we evaluated the *C. DIFF* COMPLETE in comparison with the other diagnostic methods including *C. difficile* toxigenic culture, a GDH detection test [ImmunoCard *C. difficile* (Meridian Bioscience, USA) (ImmunoCard)], and a toxin A/B detection test [TOX A/B QUIK CHEK (TECHLAB, USA) (TOX A/B)].

Materials and methods

Sample collection

A total of 60 fecal specimens were collected from 60 patients, who were hospitalized at Tokyo Metropolitan Geriatric Hospital (Tokyo, Japan) between October 2009 and January 2010 and diagnosed as having antibiotic-associated diarrhea (33 men and 27 women; age range, 54–100 years; mean, 82.9 years). All the patients consented to have their specimens used in this study. All samples examined were stored at 4°C for <72 h until *C. difficile* testing was performed.

Detection of the GDH and toxin A/B in fecal specimens

Simultaneous detection of the GDH and toxin A/B by *C. DIFF* COMPLETE, GDH detection by ImmunoCard, and toxin A/B detection by TOX A/B were performed in accordance with the manufacturers' instructions.

C. difficile culture

For *C. difficile* culture, fecal specimens were inoculated onto cycloserine–cefoxitin–mannitol agar (CCMA) (Nissui Pharmaceutical, Tokyo, Japan) and incubated for 48 h at 35°C under anaerobic conditions. The presence of the toxin A and toxin B genes of the recovered isolates was determined by PCR assay as previously described [13, 14]. For specimens that were "GDH-positive and toxin A/B-negative" by *C. DIFF* COMPLETE and "positive" by *C. difficile* culture, an additional *C. DIFF* COMPLETE test was performed on the isolates. Isolated colonies cultured on GAM agar (Nissui Pharmaceutical) for 48 h at 35°C under anaerobic conditions were suspended in the dilution buffer with turbidity equivalent to the McFarland standard no. 4 and tested by *C. DIFF* COMPLETE in accordance with the manufacture's protocol for fecal specimen testing.

Statistical analyses

As a significance test, the chi-square test with Yates' correction was performed. The significance level was set at $P < 0.05$.

Results

Clostridium difficile was isolated from 30 of the 60 fecal specimens. The GDH antigen portion of the *C. DIFF* COMPLETE test showed positive results in all 30 culture-positive specimens and negative results in 28 of the 30 culture-negative specimens, implying 100% sensitivity and 93.3% specificity. The negative predictive value was 100%. ImmunoCard exhibited positive results in 24 of the 30 culture-positive specimens and negative results in the remaining 36 specimens, indicating that its sensitivity and specificity were 80.0% (vs. *C. DIFF* COMPLETE, $P < 0.05$) and 100%, respectively (Table 1).

Table 1 Comparison of performance results for *C. DIFF* COMPLETE (GDH portion) and ImmunoCard compared to *Clostridium difficile culture*

		C. difficile culture		Sensitivity %	Specificity %	PPV %	NPV %	Correlation %
		Positive	Negative					
C. DIFF COMPLETE (GDH portion)	Positive	30	2	100 (30/30)	93.3 (28/30)	93.8 (30/32)	100 (28/28)	96.7 (58/60)
	Negative	0	28	*	**	**	**	**
ImmunoCard	Positive	24	0	80.0 (24/30)	100 (30/30)	100 (24/24)	83.3 (30/36)	90.0 (54/60)
	Negative	6	30					

PPV, positive predictive value; NPV, negative predictive value

* $P < 0.05$, chi-square test with Yates' correction

** Not significant, chi-square test with Yates' correction

The results of toxin A/B detection by *C. DIFF* COMPLETE showed a correlation of 91.7% (55/60) with those by TOX A/B (data not shown). Compared with the culture results of toxin B-positive (i.e., A^+B^+ or A^-B^+) *C. difficile* (toxigenic culture), the sensitivity and specificity of the toxin A/B portion of *C. DIFF* COMPLETE were 78.6 and 96.9%, respectively, whereas those of TOX A/B were 71.4 and 93.8%, respectively (Table 2).

Of the 30 *C. difficile* isolates recovered from stool specimens, 19, 9, and 2 isolates were A^+B^+, A^-B^+, and toxin A-negative, toxin B-negative (A^-B^-) strains, respectively. Of the 19 fecal specimens from which A^+B^+ strains were isolated, 16 and 14 specimens exhibited positive results in the toxin A/B portion of *C. DIFF* COMPLETE and TOX A/B, respectively. Similarly, of the nine fecal specimens from which A^-B^+ strains were isolated, six specimens each exhibited positive results in respective assays, and of the two fecal specimens from which A^-B^- strains were isolated, one specimen gave a positive result in both tests (results of *C. DIFF* COMPLETE are shown in Table 3).

C. DIFF COMPLETE showed "dual positives" for GDH and toxin A/B in 23 fecal specimens. Toxin B-positive *C. difficile* strains were isolated from 22 of the 23 specimens, indicating a positive predictive value of 95.7% (an A^-B^- strain was isolated from the remaining 1 specimen). *C. DIFF* COMPLETE showed "dual negatives" for GDH and toxin A/B in 28 fecal specimens. All were negative for *C. difficile* culture, implying a negative predictive value of 100%. Of the nine specimens that showed "GDH-positive and toxin A/B-negative" results by *C. DIFF* COMPLETE, seven were positive for *C. difficile* culture (Table 3). By an additional *C. DIFF* COMPLETE test on the isolates, all the six toxin B-positive isolates (three A^+B^+ and three A^-B^+ isolates) showed "GDH-positive and toxin A/B-positive" results, whereas the one A^-B^- isolate indicated a "GDH-positive and toxin A/B-negative" result (data not shown).

Discussion

In this study, we evaluated *C. DIFF* COMPLETE, a new EIA-based rapid diagnostic tool for simultaneous detection of GDH and toxin A/B in a single device.

Using *C. difficile* culture as a reference, the GDH portion of the *C. DIFF* COMPLETE had a sensitivity of 100% (30/30), which was significantly higher than the sensitivity of 80% (24/30) observed in the ImmunoCard test. GDH detection in the *C. DIFF* COMPLETE test is mediated by anti-GDH polyclonal antibodies immobilized on the membrane (capture antibodies) and enzyme-labeled anti-GDH monoclonal antibodies (detector antibodies), whereas the ImmunoCard test utilizes anti-GDH polyclonal antibodies as both capture and detector antibodies. Such differences might contribute to the high sensitivity and specificity of GDH detection in *C. DIFF* COMPLETE.

For two specimens that were GDH positive by *C. DIFF* COMPLETE but culture negative, we cannot exclude the possibility of false-negative results of *C. difficile* culture. However, it was more strongly suspected that the GDH detection gave false-positive results because they exhibited negative results in all the other tests performed (i.e., GDH detection by ImmunoCard, toxin A/B detection by *C. DIFF* COMPLETE and TOX A/B), and neither of the two

Table 2 Comparison of performance results for *C. DIFF* COMPLETE (toxin portion) and TOX A/B compared to toxigenic culture

		A^+B^+/A^-B^+[a] *C. difficile* culture		Sensitivity %	Specificity %	PPV %	NPV %	Correlation %
		Positive	Negative					
C. DIFF COMPLETE (toxin portion)	Positive	22	1	78.6 (22/28)	96.9 (31/32)	95.7 (22/23)	83.8 (31/37)	88.3 (53/60)
	Negative	6	31		**	**	**	**
TOX A/B	Positive	20	2	71.4 (20/28)	93.8 (30/32)	90.9 (20/22)	78.9 (30/38)	83.3 (50/60)
	Negative	8	30					

PPV, positive predictive value; NPV, negative predictive value

** Not significant, chi-square test with Yates' correction

[a] A^+B^+, toxin A-positive, toxin B-positive; A^-B^+, toxin A-negative, toxin B-positive

Table 3 Results of the *C. DIFF* COMPLETE test and *C. difficile culture*

C. DIFF COMPLETE detecting		*C. difficile* culture			
		Positive			Negative
GDH	Toxin A/B	A^+B^+[a]	A^-B^+	A^-B^-	
Positive	Positive[b]	16[c]	6	1	0
Positive	Negative	3	3	1	2
Negative	Positive	0	0	0	0
Negative	Negative[b]	0	0	0	28
Total		19	9	2	30

[a] A^+B^+, toxin A-positive, toxin B-positive; A^-B^+, toxin A-negative, toxin-B-positive; A^-B^-, toxin A-negative, toxin B-negative

[b] A positive predictive value of "GDH-positive and toxin A/B-positive" results and a negative predictive value of "GDH-negative and toxin A/B-negative" results were 95.7% (22/23) and 100% (28/28), respectively

[c] Number of stool specimens

patients had received vancomycin or metronidazole for CDI before sample collection.

The toxin A/B portion of *C. DIFF* COMPLETE was able to detect both A^+B^+ and A^-B^+ strains, and had a detection rate of 78.6% (22/28) compared with the results of toxigenic culture, which was slightly higher than the rate of 71.4% (20/28) observed in the TOX A/B test (the difference was not statistically significant). Two A^+B^+ strains showed positive results in *C. DIFF* COMPLETE but negative results in TOX A/B, whereas the detection rate for A^-B^+ strains was the same for both tests (six of the nine specimens indicated positive results in each test).

Rapid detection EIA tests for toxin A/B have been reported to lack sufficient sensitivity [5–7]. In the present study, the toxin A/B portion of *C. DIFF* COMPLETE showed a relatively low sensitivity (78.6%), whereas the GDH portion indicated a sensitivity of 100%. Recent studies on *C. DIFF* COMPLETE have also described a low sensitivity of the toxin A/B portion (61.1–78.3%), in contrast to the high sensitivity of the GDH portion (97.6–100%) [10–12]. The specificity of toxin A/B detection by *C. DIFF* COMPLETE was high (96.9%), as reported in the articles described above (99.2–100%). Moreover, our study revealed a negative predictive value of 100% in GDH detection. Given such excellent sensitivity and negative predictive value of the GDH portion and the high specificity of the toxin A/B portion, using *C. DIFF* COMPLETE as the first-line screening test for CDI is considered reasonable.

Toxin B-positive *C. difficile* strains were isolated from 22 of the 23 fecal specimens that showed "dual positives" for GDH and toxin A/B by the *C. DIFF* COMPLETE test, implying a positive predictive value of 95.7%. The remaining 1 specimen from which an A^-B^- strain was isolated might have contained both toxin B-positive and A^-B^- strains, because TOX A/B as well as *C. DIFF* COMPLETE exhibited positive results for toxin A/B in this specimen. *C. difficile* culture was negative in all the 28 specimens that showed "dual negatives" for GDH and toxin A/B by the *C. DIFF* COMPLETE test, indicating a negative predictive value of 100%. Thus, results showing "dual positives" for GDH and toxin A/B strongly suggest "true positive," i.e., the presence of toxin B-positive

C. difficile strains in feces, whereas results showing "dual negatives" imply "true negative."

When the *C. DIFF* COMPLETE test shows "GDH-positive and toxin A/B-negative" results, it cannot be concluded that the nontoxic (A^-B^-) strain of *C. difficile* exists in feces. However, the GDH-positive result may alert clinicians to the possibility of the presence of toxigenic organisms as a result of the false-negative result for toxin A/B. Performing confirmatory tests only for the specimens with discrepant results (not for the dual-positive and dual-negative specimens) would alleviate the burden imposed on clinical microbiology laboratories and help improve the efficiency of accurate CDI diagnosis. Confirmatory testing by PCR on stool samples allows a rapid, simple, and accurate diagnosis [10–12], but the initial expenditure for purchase of the instrument is considerable. A cytotoxin assay may be also impracticable for many clinical laboratories because of the need for special equipment and techniques. Some researchers evaluated a combination of a toxin detection kit and *C. difficile* culture, in which the toxigenicity of a culture isolate was retested with the toxin detection kit [15, 16]. Such a diagnostic strategy may be feasible in many clinical institutions, although several days are required before results are reported.

C. difficile is important as a pathogen that frequently causes healthcare-associated infections. In the present study, 28 inpatients were diagnosed as having CDI during the study period of approximately 3.5 months. It is strongly suspected that some strains spread among these patients, although typing analysis on recovered isolates was not performed in this study. Diagnostic algorithms, utilizing the *C. DIFF* COMPLETE as an initial screening test, may contribute to the infection control of CDI by allowing us to make more accurate and rapid diagnosis of the infection.

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＜特別寄稿＞

B型肝炎universal vaccinationへ向けて

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要旨：B型肝炎ワクチンは諸外国では乳児期に全員が接種を受けるユニバーサルワクチンである．しかしながら我が国では任意接種（セレクティブワクチネーション）となっており，母児感染防止の場合のみワクチン接種が健康保険でカバーされている．

こうしたセレクティブワクチネーションのみでは我が国のB型肝炎を制圧することは困難である．

本稿では平成23年6月2日に第47回日本肝臓学会（小池和彦会長）において行われたワークショップ「B型肝炎universal vaccinationへ向けて」の内容を紹介しながら，ユニバーサルワクチネーションに関してまとめてみたい．

索引用語：　セレクティブワクチネーション　　母子感染　　水平感染
De novo肝炎　　HBV Genotype

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＜受付日2011年10月12日＞＜採択日2011年12月29日＞

1　B型肝炎の感染防止策

1）我が国における感染防止策

1972年にHBs抗原の検査が広く行われるようになり，B型慢性肝疾患の自然史や実態が明らかにされていった．慢性肝炎の症例には家族集積性があることが以前からわかっていたが，その多くがHBs抗原陽性であることが判明した[1)]．また，B型肝炎ウイルスキャリア妊婦から生まれた児の20～30％が持続感染へ移行することが明らかにされた[2)]．このため，我が国のB型肝炎対策の大きな柱は垂直感染の遮断に置かれることになった．

1975年，HBs抗原陽性の妊婦から生まれた児が高率に持続感染に移行すること，こうした症例の多くは胎内ではなく出生時に感染することが明らかにされた[3)]．また，持続感染に移行する症例のほとんどがHBe抗原陽性であり，HBe抗原陰性の場合は一過性感染を起こす場合はあるがキャリア化することは稀であることも判明した[4)]．このため，HBe抗原陽性の母親から生まれた児を対象に高力価抗HBsヒト免疫グロブリン(HBIG)を用いてHBs抗原陽性の母親からの垂直感染を防御しようとする試みが行われた．HBIGを約1年間反復投与している間は児はHBs抗原陰性であったが，中止するとやがてHBs抗原陽性となる児が生じ，受動免疫だけ

では児のHBVキャリア化を完全には防ぐことができないことが明らかになった[5][6]．

1984年に国産のHBワクチンによる能動免疫が使用可能となり，HBIGとの併用による感染防御が行われるようになった[7]．1985年には厚生省「B型肝炎母子感染防止事業」が開始され，HBe抗原陽性の母から1986年1月1日以降出生した児に対して全国的にHBIGとHBVワクチンによるB型肝炎母子感染防止が始まった（1995年に保険診療で予防を行うようになった時点で，対象はHBe抗原陽性/陰性を問わずすべてのHBs抗原陽性の母親に拡大された）．この事業実施前後のHBs抗原陽性率の推移がいくつか報告されているが，どの報告でも母子感染は大きく減少している[8]~[11]．また，小児のB型肝細胞癌の発生が減少したことも最近報告された[12]．これら一連の成果は我が国の基礎研究者，内科医，産婦人科医，小児科医の連携が生んだものであり，我が国の誇るべき成果である．

2）台湾における感染防止策

台湾のHBs抗原陽性率は日本と比べてはるかに高く（15～20%），感染率を低下させることが急務であった．このため，HBs抗原陽性の母親から生まれた新生児を対象に1984年にHBワクチンが導入された[13]．1986年には接種の対象がすべての新生児に拡げられた．ワクチン接種の対象は徐々に拡げられ，1991年には20歳未満のすべての国民がワクチン接種の対象となった．さらにHBs抗原陽性の母親から生まれた児には，出生時にHBIGの投与が行われるようになった[14]．

その結果，ユニバーサルワクチネーション導入10年後には，小学1年生（6歳）のHBs抗原陽性率が10.5%から1.7%にまで低下した[14]．また，6歳から14歳の子供の10万人当たりの肝細胞癌の発生率は，1981～1986年の期間の0.70から1990～1994年の0.36へと短期間で大幅に減少した[15]．

3）アメリカにおける感染防止策

アメリカではHBワクチンが1981年から使われるようになったが，当初は職業感染対策として使用されていた．1991年Advisory Committee on Immunization Practices（ACIP）のImmunization Practices Advisory Committeeからレコメンデーションが出され，HBs抗原陽性の妊婦から生まれた児に対してHBIGとHBワクチンを併用することが定められた[16]．その後すべての新生児，19歳未満の青少年，ハイリスク集団と段階的に接種対象が拡げられていった．その結果2005年には，19歳未満の子供及び青少年の感染率は96%減少した．

しかしながら19歳以上では76%の減少に留まった[17]．これは成人のワクチン接種率が低いためと考えられた．

4）その他の国における感染防止策

イタリア，中国，タイ，ガンビアなどはユニバーサルワクチンを早い時期から導入してきた国である．こうした国では必ずしも高価なHBIGを使用できたわけではないが，国民のHBV感染率を70%程度減少させることに成功している．乳幼児期以降の水平感染の防止効果と思われる[18]．こうした事実もふまえ，WHOは出産時の母児感染予防としてユニバーサルHBワクチンを推奨している[19]．1992年にはすべてのWHO加盟国に対して，1997年までにB型肝炎ワクチンをExpanded Program on Immunization（EPI：予防接種拡大プログラム）に組み入れるよう勧告が出されている[20]．

2　セレクティブワクチネーションとユニバーサルワクチネーション

2007年の段階でWHOの勧告を受け入れず，セレクティブワクチネーションを行っているのは，先進国ではヨーロッパの7カ国（デンマーク，フィンランド，アイスランド，オランダ，ノルウエー，スウェーデン，イギリス）と日本のみである．アイルランドは国民のHBVキャリア率が2%未満であったが，移民の増加やSTDや旅行に伴う感染者の増加を契機に，経済効果も考慮に入れた上で1998年からユニバーサルワクチンを導入した[21][22]．

セレクティブワクチネーションは，B型肝炎に感染する可能性が高い人のみを対象としてワクチン接種を行う方法である．これまでの疫学的事実から，国民のHBs抗原陽性率が中程度（2%から8%）以上の場合には，国民全員を対象にしたユニバーサルワクチネーションを採用した方が効果的とされている．また，国民のHBs抗原陽性率が低い（2%未満）場合は，ユニバーサルワクチネーションの他，ハイリスクグループを対象にしたセレクティブワクチネーションも効果的とされている[23]．

ハイリスクグループには様々な集団がある．どのような集団がヨーロッパ7カ国でセレクティブワクチネーションの対象にされているかをTable 1に示した．母子感染防止以外に多くのハイリスクグループが公費助成の対象とされていることがわかる．特にB型肝炎ウイルスキャリアの同居家族や静脈注射常習者（IV drug

Table 1　Selective vaccination 実施国におけるワクチン接種対象者

	職業上のリスク*	家族接触†	キャリア妊婦からの出生児	養護施設，介護施設の患者や職員	免疫不全患者や頻回輸血が必要な患者‡	ハイリスク集団§	両親が高または中浸淫地域出身の子供$	HBキャリアがいる保育園（託児所）の園児	職業ではないが針刺しや血液への曝露の危険性がある人
デンマーク	Yes	Yes	Yes	Yes	No	Yes	No	No	No
フィンランド	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes
アイスランド	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
アイルランド	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
オランダ	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes**
ノルウェー	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
スウェーデン	Yes	Yes	Yes	Yes	Yes	Yes	Yes（2005-）	Yes	No
英国	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
日本	No	No	Yes	No	No	No	No	No	No

＊医療関係者，警察などの警備関係者，救急消防関係者，高浸淫地域で働く予定の学生，性風俗労働者．フィンランドではHBVワクチン接種は雇用者に責任がある．
†B型急性肝炎，B型慢性肝炎患者の配偶者，sexパートナー，家族，同居人
‡慢性腎不全，慢性肝炎，血友病患者
§薬物常用者のsexパートナー子供/同居人，MSM（men who have sex with men），複数のsexパートナーを有する人，囚人，刺青芸術家，高浸淫地域からの移民または高浸淫地域への旅行者
$高浸淫地域からの移民の子ども，高浸淫地域からの養子とその教育者を含む家族

（文献69を一部改変）

user），MSM（men who have sex with men）はすべての国で公費助成の対象とされている[22]．日本の公費助成（健康保険の適応）の対象がキャリア妊婦に限られているのとは大きな差がある．

このように日本はこれまで少ない費用で大きな効果を上げてきた世界でも希有な国である．その理由は母子感染防止事業の実施率が極めて高かったからと推測される．その背景には，ほとんどの出産が医療機関で行われること，産婦人科医/小児科医に母子感染防止事業の遵守が徹底したこと，乳児へのワクチン接種に家族が熱心であったこと，国民の衛生に対する意識が高かったことなどがある．

3　B型肝炎の水平感染防止は重要である

1）乳幼児期の水平感染防止

乳幼児期のB型肝炎への感染に関してはこれまで長期にわたり検討が行われてきた．母子垂直感染の標準予防処置に関してはよく守られていることが本ワークショップでも田尻（仁）らにより報告された．従って母子感染阻止失敗例の多くは，妊娠中の母親の感染を主因とする胎内感染である．

その一方で父親を中心とする同居家族からの感染が母子感染阻止失敗例とほぼ同数認められる[24)~26)]．B型肝炎ウイルスキャリアの体液には感染性を持つHBVが含まれることが今回小松らにより明らかにされたが，父子感染を含めた家族内感染の大きな原因と思われる．

以下に述べるように本邦のB型急性肝炎の主体はGenotype Aに代わりつつあり[27]，Genotype AのHBVキャリアに占める割合も増加傾向にある[28)29)]．これはヨーロッパにおける持続感染の95％は水平感染によるという事実と整合する[30]．今後は本邦でもGenotype AのHBVキャリアから乳幼児への水平感染が問題になる可能性がある．

前述の通りB型肝炎ウイルスキャリアの同居家族は海外では公費助成の対象となっており，本邦においても早急に検討が必要である．また，保育施設等での水平感染も乳幼児期の感染の原因として問題となる[31]．

2）STD（sexually transmitted diseases）としてのB型急性肝炎の予防

現在思春期以降のB型急性肝炎のほとんどは性交渉によって伝播する．性風俗の多様化に伴い，不特定多数の異性との性交渉，男性間での性交渉が増えてきており，半数以上を占めている[27]．

現在のB型急性肝炎罹患者（無症状の者も含む）は年間約11,000人程度と推定されている（伊藤）．症状を有するB型急性肝炎に占めるGenotype Aの割合は増

加傾向にあるが[27]，今回のワークショップでも症状を有するB型急性肝炎の約半数がGenotype Aの感染によることが，複数の演者（伊藤，柘植，中西，林）から報告された．従って不特定多数の異性との性交渉，男性間での性交渉によるB型急性肝炎（その多くがGenotype A）は年間3,000人以上を占めるものと推定される．

旧国立病院機構の調査によればB型急性肝炎の約2%が劇症化するとされているが[32]，現在もなおB型急性肝炎からの劇症化例，死亡例があることが梅村ら，松田らによって報告された．また，Genotype AのB型急性肝炎の1割前後が遷延，慢性化することが明らかにされつつあるが[33]，今回のワークショップでもその傾向は同じであることが，梅村，伊藤，柘植の発表で明らかにされた．Genotype AのB型慢性肝炎の自然史には不明な点もあるが，肝硬変/肝細胞癌に至る症例の割合はヨーロッパと東アジア諸国と大差はない[34]こと，Genotype AとDの進展肝疾患の合併率に大きな差がないこと[35]を考えると肝硬変/肝細胞癌に進展する例が数%存在することは間違いないと思われる．

こうした実態を考えるとSTDとしてのB型急性肝炎をワクチンで予防することは重要なことである．海外からはSTDとしてのB型急性肝炎の予防には11-12歳でのユニバーサルワクチンが効果的であるとの報告がある．奥瀬らが発表したようにこの年代層の抗体獲得率は乳児期同様高い[36]．また，HPVワクチンの接種は現在，主として中学1年生から高校1年生の女子に対し行われている．今後本邦でも中学生から高校生を対象にしたHBワクチンの接種を検討する必要があろう．

3）De novo肝炎の予防

リツキシマブが使用されるようになって以来，B型肝炎既感染例からのHBV再活性化が数多く報告されるようになった．本邦の前向き調査ではB型肝炎既感染例の12%に再活性化が見られると報告されており[37]，さらに大規模な全国調査も現在名古屋市立大学を中心として進行中である．リツキシマブを使用する化学療法の際には日本肝臓学会から出された“免疫抑制・化学療法により発症するB型肝炎対策ガイドライン”[38]が適用されることが多く，重症化例の報告は見られなくなっているが，検査及び投薬に多額の費用がかかっている．また，TNF-α阻害薬など分子標的薬による再活性化の報告も最近増えてきている[39)~41)]．

こうした再活性化の防止のために，最も効率的な手段はHBワクチンによりB型肝炎ウイルスへの感染そのものを阻止することである．

4）輸血後肝炎の予防

現在輸血時のB型肝炎スクリーニングは20プールNAT（Nucleic Acid Amplification Test）で行われている．この方法ではウインドウ期（感染後HBV DNAが陽性となるまでの時期）や肝炎治癒後に血中に微量に存在するウイルスを検出できない場合があり，現在も年間10例程度の輸血後B型肝炎が発生している（http://www.jrc.or.jp/vcms_lf/iyakuhin_yuketuj1010-125_101029.pdf）．輸血後肝炎の防止のためにはHBワクチンによりB型肝炎ウイルスに対する免疫を獲得しておくことが最も有効である．

4　基調講演の内容

今回のワークショップでは国立成育医療研究センター感染症科，米国感染症専門医の齊藤昭彦先生による基調講演が行われた．その内容は以下の通りである．

（1）アメリカをはじめ，先進国をはじめとした多くの国々ではユニバーサルHBワクチンが導入されているが，日本はHBワクチンを含め，定期接種に組み入れられていない重要なワクチンが他の先進国と比べて多い．

（2）アメリカではワクチンの安全性，効果をACIP（Advisory Committee on Immunization Practices）が政府と独立して科学的，客観的事実に基づいて評価しており，スケジュール等の決定に大きな役割を果たしている．日本でもこのような機関の設置が望まれる．

（3）ワクチンで防御可能な感染症に関しては，集団の接種率を上げることによって社会全体がその感染症に免疫を持ち（集団免疫，Herd Immunity），予防接種が不完全あるいは不可能な新生児・乳幼児や高齢者，基礎疾患を持つ人などを守ることができる．この考えはHBワクチンが海外でユニバーサルワクチンとなっている大きな理由である．

（4）日本小児科学会は本年学会として勧奨するワクチンスケジュール（http://www.jpeds.or.jp/saisin/saisin_110427.pdf）を公表した．この中ではHBワクチンは“乳児期に他のワクチンと同時接種を行うべき”ワクチンと位置づけられている．また，乳幼児期の接種を行っていない10歳以上の学童に対しては，水平感染予防のためのHBワクチンを日本小児科学会として推奨すると明記されている．

5　ワークショップで討議が行われたその他の問題

1）HIV/HBV重複感染

B型肝炎の感染経路，MSMが増加しつつあることを

考えると，B型急性肝炎にはHIV感染症を合併する可能性がある．柘植，高橋（祥一）らはB型急性肝炎34例中7例（21%）がHIVとの重複感染だと発表した．また，世古口らはHIV感染症患者59名中6名がHBs抗原陽性であることを発表した．B型急性肝炎の新規症例に対しては患者の了承を得てHIVスクリーニング検査を行うことが望ましい．なお，HIV合併例に対するエンテカビル投与は，HIV薬剤耐性を誘発する可能性が高いので，単独での使用は避けるべきであることがガイドラインで明記されている（http://www.hivjp.org/guidebook/hiv_14.pdf）．

2）慢性化の予知は可能か？

B型急性肝炎の慢性化を予知し，阻止することが今後重要である．高橋（秀明）らはHBs抗原及びHBcr抗原の定量で予測がある程度可能であることを発表した[33]．

3）現在のワクチンで異なるGenotypeのHBV株やワクチンエスケープ変異株の感染防御は可能であるか

菅内らはキメラマウスを用いたin vivo系で，田尻（和人）らはin vitroの細胞培養系で検討を行い，Genotypeの異なるHBV株やワクチンエスケープ変異株の感染防御は可能であるとの成績を示した[42]．ただし，特に後者に関しては比較的高い抗体価が必要である．抗体価が低い場合はウイルスの再感染を完全には防御できないことを示唆する成績も出されており[43]，ワクチン接種により十分な抗体価を得ることが望ましい．肝移植のレシピエントの肝内ではエスケープ変異株が高率に検出されることが今回発表され（上田），水平感染の可能性も含め今後の検討が必要と思われた．

4）HBIGに関して

HBIGは血液製剤であり，製造にはHBs抗体価が高力価陽性の供血者が必要である．このような供血者を確保するにはワクチン接種者の中でHBs抗体の反応が良好な方の協力が必要不可欠であるが，ワクチン接種者が少ない現状では供血者の確保が困難になりつつある．八橋らは厚生労働省班研究の一環として，HBs抗体陽性例にHBワクチン投与し，HBs抗体力価を上昇させ献血に協力いただくという積極的，能動的収集を日本赤十字社との共同研究で始めているが，非常に重要なHBIGの供給源となる可能性がある．

6　日本肝臓学会役員・評議員に対するアンケートの結果

2009年のワークショップ「ユニバーサルHBワクチン：是か非か？」に先立ち，日本肝臓学会役員・評議員を対象にしたアンケートが行われ，結果が公表されている[44]．今回も同様のアンケートを実施した．アンケート項目をTable 2に示す．回収率は全体で40%（86/214）であった．

質問1：ユニバーサルワクチンの導入に賛成かどうか

賛成98%，どちらでもない2%，であった．

質問2：ユニバーサルワクチンの接種対象者はどうすべきか

乳幼児及び青少年73%，乳幼児のみ21%，青少年のみ6%，であった．

質問3：（乳幼児及び青少年と回答した場合）乳幼児，青少年のどちらから開始するか

同時に開始する61%，青少年から23%，乳幼児から16%，であった．

質問4：乳幼児の接種時期の変更は必要か

必要である45%，どちらでもよい37%，必要ない16%，その他2%，であった．

質問5：青少年へ接種する場合その時期はいつが適当か

12歳69%，15歳27%，その他4%，であった．

質問6：ワクチン接種で陽性となったHBs抗体の力価が低下した場合，ワクチンの追加投与（ブースター）は必要か

不要である44%，どちらとも言えない37%，必要である19%，であった．

その他の意見

＊抗体陽性化した症例に対する数年後ブースターの是非（感染を防ぐのか肝炎を防ぐのか）を明確にしてほしい．

＊UVの導入には諸家の意見があると思うが，日本では垂直感染予防プログラムが奏効していること，幼児期の水平感染は少ないと考えられること，青年期以降のゲノタイプAの浸淫はきわめて危惧すべき状況であること，などから，まずは青年期から開始して，次に，その必要性を吟味した上で，乳幼児期へ拡大する2段階法で十分ではなかろうか．

＊麻疹ワクチンの接種でも判明したが，思春期は子宮頸癌ワクチンのようなインパクトのあるキャンペーンをはらないと良好な接種率は得られないと思う．従って，あらゆる手段で啓発するとともに，青年期は小6での接種が最も効果的と考える．

＊乳幼児期にワクチン接種→青少年期に抗体価チェック→陰性であればブースターワクチン接種，というのはいかがであろうか？