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Evaluation of a New Anti-HCV-Assay Kit for Anti-HCV Screening in Early Childhood

KOHACHIRO SUGIYAMA, KENJI GOTO, YOSHISHIGE MIYAKE, FUMIYA MIZUTANI, KOJI TERABE, ROU LI, HIROKO MANIWA, YOSHIKAZU KAWABE and YOSHIRO WADA

Department of Pediatrics, Nagoya City University Medical School, Nagoya 467-8601

SUGIYAMA, K., GOTO, K., MIYAKE, Y., MIZUTANI, F., TERABE, K., LI, R., MANIWA, H., KAWABE, Y. and WADA, Y. *Evaluation of a New Anti-HCV-Assay Kit for Anti-HCV Screening in Early Childhood.* Tohoku J. Exp. Med., 1999, **187** (3), 257-262 —— Sera of 20 children falsely identified as positive for hepatitis C virus antibody (Anti-HCV) by a second generation anti-HCV-assay kit (Imucheck-HCV Ab "Kokusai") were re-tested using a new third generation anti-HCV-assay kit (Imucheck • F-HCV C50 Ab "Kokusai"). Seventeen of the samples were reclassified as negative and only three remained positive. Changing well solids in the anti-HCV-assay kit from casein to bovine serum albumin appears to have improved the false-positive rate, most likely as a result of decreased non-specific adsorption of casein antibodies. ———— Anti-HCV; false positive; casein antibodies

Since the first generation anti-HCV-assay kit that used c100-3 antigen derived from the NS4 region of HCV was developed in 1989 (Kuo et al. 1989), various types of anti-HCV-assay kits have been marketed. At present, third generation anti-HCV-assay kits that utilize recombinant antigens or synthetic peptide antigens derived from the core, NS4 and NS5 regions of HCV are commercially available. These kits are useful for screening HCV infected adults (McHutchison et al. 1992; Uyttendaele et al. 1994). However, effective HCV screening in early childhood using these kits remains a problem (Maniwa et al. 1997). Recently, a new anti-HCV-assay kit using an epitope chimeric antigen (C50), Imucheck • F-HCV C50 Ab "Kokusai" (International Reagents Co., Tokyo) (Yagi et al. 1996), has been introduced in Japan. In the present study, we evaluated the efficiency of this new anti-HCV screening kit in early childhood using samples falsely identified as positive by an older kit, Imucheck-HCV Ab "Kokusai".

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Address for reprints: Kohachiro Sugiyama, Department of Pediatrics, Nagoya City University Medical School, Kawasumi-cho, Mizuho-ku, Nagoya 467-8601, Japan.
e-mail: sugiyama@med.nagoya-cu.ac.jp

TABLE 1. *Anti-HCV determination by Imucheck-HCV Ab/muchek•F-HCV and HCV RNA presence/absence by RT-nested PCR*

Case (No.)	Age (months)	Imu-check	Imu-check•F	Imu-check•F C50	Immuno-judge		
					c7	c11	HBG
1	3	3.6[a]	1.91[a]	3.45[a]	0[b]	0[b]	M
2	8	1.40	0.26	0.30	0	0	
3	8	3.90	3.28	0.38	0	2	
4	9	3.84	0.50	0.54	0	0	W
5	10	2.09	0.51	0.29	±	0	W
6	10	1.17	0.08	0.07	0	0	
7	11	1.20	0.60	0.75	±	±	
8	11	1.20	1.25	1.44	±	±	
9	11	1.50	0.10	0.09	0	0	
10	15	2.45	0.31	0.35	0	0	W
11	17	2.35	0.33	0.38	0	0	W
12	17	1.78	0.16	0.18	0	0	W
13	21	3.20	0.37	0.30	0	0	W
14	24	1.50	0.61	0.72	0	0	W
15	32	2.20	0.28	0.31	0	0	
16	52	1.60	1.34	0.02	1	0	
17	63	1.10	0.05	0.06	0	0	
18	92	4.10	0.39	0.59	0	0	
19	10	4.19	1.23	1.01	0	0	W
20	37	2.10	0.74	0.87	0	0	
21	5	7.85<	10.14	4.72	2	2	
22	6	7.80<	16.91	25.27	±	2	
23	68	9.62<	60.05	50.35	4	4	
24	97	7.90<	75.00<	54.50<	4	4	
25	112	7.85<	75.00<	54.50<	2	4	
26	142	7.83<	75.00<	54.50<	4	4	
27	145	7.85<	75.00<	54.50<	4	4	
28	168	7.80<	75.00<	54.50<	4	4	

[a]COI value of anti-HCV, [b]strength of band coloring on strips.
NT, not tested; −, negative; +, positive.

MATERIALS and METHODS

Sera from 1000 children who visited the Pediatric clinic at the Nagoya City University were screened for anti-HCV using the Imucheck-HCV Ab kit (contained c7/c11 antigens and casein in well solids). Sera of 28 of these children, which were initially identified as positive for anti-HCV, were reevaluated by enzyme-linked immunosorbent assay (ELISA) using the Imucheck•F-HCV Ab

Ab, Imucheck • F-HCV C50 Ab, Immunojudge, and Immunojudge C50 kits,

Immunojudge C50						RNA
NS4-2	NS4-1	NS3	Core	C50	HBG	
0[b]	0[b]	0[b]	0[b]	0[b]	M	−
0	0	0	0	0		−
0	0	0	±	3		−
0	0	0	0	±	M	−
0	0	0	0	0	W	−
0	0	0	0	0		−
0	0	±	±	1		−
0	0	±	±	1		−
0	0	0	0	0		−
NT	NT	NT	NT	NT	NT	−
0	0	±	0	±	M	−
0	0	0	0	0	W	−
0	0	0	0	0	W	−
0	0	0	0	0	W	−
0	0	0	0	0		−
0	0	0	0	0		−
0	0	0	0	0		−
0	0	0	0	0		−
0	0	±	0	0		−
0	0	0	0	0	W	−
0	0	0	1	2		+
2	1	±	4	4		+
0	±	4	3	4		+
4	4	4	4	4		+
4	1	4	4	4		+
0	1	4	4	4		+
2	4	3	4	4		+
0	2	4	4	4		+

HBG, homogeneous coloring of background; M, moderate; W, weak;

"Kokusai" (intermediate kit; contained c7/c11 antigens and bovine serum albumin in well solids) and Imucheck • F-HCV C50 Ab (contained C50 antigen and bovine serum albumin in well solids) kits. Samples with a cut off index (COI) of 1.0 or higher were determined to be positive, and samples with a COI below 1.0 were considered negative. Furthermore, the anti-HCV-positive samples were tested by immunoblot assay using the Immunojudge (Tonen Co., Tokyo) and Immunojudge C50 (Tonen Co.) kits. In these immunoblot assays, the intensity of

bands coloring the strip was assessed. Gradations of 0, ±, 1, 2, 3 and 4 were adopted as an assessment standard, and the presence of color ranked 1 or higher was deemed positive. All assays were performed according to the manufacturer's instructions. HCV RNA in the sera was determined using the reverse transcriptase-polymerase chain reaction as previously described (Li et al. 1997).

All serum samples were stored at −30°C until use. Parental consent was obtained for all patients before blood samples were drawn.

Results

Among 28 children who were identified as anti-HCV positive by the Imucheck-HCV Ab kit, eight (Cases 21–28; COI values >7.8) were positive for serum HCV RNA. The remaining 20 children (Cases 1–20), who had lower COI values (between 1.0 and 5.0), were negative for serum HCV RNA. Two of the 20 children (Cases 19 and 20), who had a history of blood transfusions due to surgery for congenital heart disease, did not have elevated alanine aminotransferase (ALT) or anti-HCV levels, and were negative for serum HCV RNA during the first years after the transfusions. Two to four months after the first test, the anti-HCV titers of the remaining 18 children were similar to or lower than those of the first test and no HCV RNA was found in their sera. Mothers of the 20 children were negative for serum anti-HCV.

Sera from all eight HCV RNA-positive children were identified as anti-HCV positive by both the Imucheck•F-HCV Ab and Imucheck • F-HCV C50 Ab kits. On the other hand, seropositivity rates of the 20 HCV RNA-negative children were 5/20 and 3/20 by the Imucheck • F-HCV Ab and Imucheck • F-HCV C50 Ab kits, respectively. Although fewer samples were identified as positive by the Imucheck • F-HCV C50 Ab kit, three of the original samples (Cases 1, 8 and 19) remained positive.

Band coloring in response to one of two antigens in the Immunojudge kit was observed in cases 3 and 16, and band coloring in response to one of five antigens in the Immunojudge C50 kit was observed in Cases 3, 7 and 8. Furthermore, in Cases 1, 4–5, 10–14, and 19–20, homogeneous coloring was observed on strips, possibly due to casein antibodies.

Discussion

The HCV Ab-assay kit, Imucheck-HCV Ab, which contains two antigens (c7/c11) derived from the structural (c11) and non-structural (c7) regions of HCV, is believed useful for screening patients with HCV infection (Saito et al. 1992). However, in a previous study we found that about 1% of young children were identified as anti-HCV positive by the Imucheck-HCV Ab kit and that most of these results, with a COI of less than 6.0, were in fact false-positives due to the presence of casein antibodies in serum (Maniwa et al. 1997). However, such low COI values are observed in both the early stage of HCV infection and in patients

cured from HCV infection. Thus, improving the anti-HCV-assay kit to decrease the false-positive rate for anti-HCV screening is a matter of paramount importance, a need which led to a change from casein to bovine serum albumin as a component of the well solids, such as in the Imucheck • F-HCV Ab kit. Subsequently, a new kit, Imucheck • F-HCV C50 Ab has been introduced, which contains an epitope chimeric antigen (C50), composed of nine major epitope regions (two in NS3, two each in the NS4 of two genotypes, two each in the core of two genotypes, and one in the core of HCV polypeptide) (Yagi et al. 1996).

Although the possibility of false negative results for serum HCV RNA remains, we believe the 20 children selected for the present study were false positives (as originally identified by the Imucheck-HCV Ab kit) based on the following: 1) ALT levels were not elevated, 2) serum HCV RNA was not detected, 3) no reaction to c7 or c11 antigen, 4) detection of homogenous background coloring due to non specific adhesion of casein antibodies in the Immunoblot assays in most cases, and 5) no history of vertical or horizontal transmission of HCV except in two cases, during the follow-up period. The major difference in components between the Imucheck-HCV Ab and Imucheck • F-HCV C50 Ab kits was a change from casein to albumin in the well solids. Thus, the decreased positive rate in the Imucheck • F-HCV C50 Ab kit is most likely due to the decrease in non-specific adsorption of casein antibodies.

Assuming no technical errors, the false positives identified by the Imucheck • F-HCV C50 Ab kit may be caused by the presence of antigen related factors i.e., cross reactive antibodies reacting to antigens or promoter proteins, or non-specific adsorption of serum components or antibodies to the non-antigen component of the well solids (bovine serum albumin). To establish the existence of antigen related factors in the three anti-HCV positive cases (Cases 1, 8 and 19), an immunoblot assay was performed using the Immunojudge C50 kit. Weak reactivity to the C50 antigen was observed only in case 8. In addition, in cases 8 and 19 (not tested in Case 1) the anti-HCV titer was measured using Imucheck • F-HCV C50 Ab buffer with (usual assay conditions) or without bovine serum albumin. The anti-HCV titer in Case 8 did not change under either condition whereas that of Case 19 decreased when analyzed using buffer that contained bovine serum albumin. Thus, the false-positive result obtained in Case 19 may have been due to the presence of antibodies to bovine serum albumin.

In a separate Imucheck • F-HCV C50 Ab screening of 200 young children without exposure to HCV infection, no positive results were obtained (personal communication, Dr. I. Watanabe, Nagoya City Johoku Hospital). Together with our results, this suggest that this new kit is useful for anti-HCV screening in young children. However, a larger number of samples must be analyzed to better assess the efficiency of the Imucheck • F-HCV C50 Ab kit.

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