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Hepatitis C Testing:

Comparison of Ortho's EIA and RIBA II Tests in 1,182 Patients Undergoing Primary Liver Transplantation

Maya Rochlani, M.D.^{1,3}, Jessica H. Lewis, M.D.^{1,2}, Glenn E. Ramsey, M.D.^{1,3}, Franklin A. Bontempo, M.D.^{1,2}, Gunjan Shah, M.D.⁴, Rebecca A. Bowman, R.N.¹, David H. van Thiel, M.D.⁴, and Thomas E. Starzl, M.D., Ph.D.⁴

¹Central Blood Bank, Pittsburgh, University of Pittsburgh, Pittsburgh, Pennsylvania.

²Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

³Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania.

⁴Department of Transplant Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania.

Abstract

Plasma samples from 1,182 patients undergoing primary liver transplantation were tested for anti-hepatitis C (HCV) virus by two methods: Ortho HCV ELISA Test System (EIA) and Chiron RIBA HCV Test System (RIBA II). The EIA results, 0 or +, were recorded first, followed by RIBA results, N = negative, P = positive, or I = indeterminate. Concordant results—0N, + P, + I—were found in 1,076 (91%), and discordant results were found in 106 (9%). The EIA optical density did not relate to concordant or discordant results. Band patterns were described by stating the band position (1, 2, 3, or 4) and inseting a dash (–) if no band was visualized. Most + P samples fell into two patterns: 47% showed all four bands, pattern 1234, and 15% showed the two-band pattern, 34. When the EIA was negative, 0P, the opposite was seen: 8% showed the 1234 pattern and 81% showed the 34 pattern. There were 226 samples that formed bands (+ P, 149; 0P, 31; + I, 15; 0I, 31). The frequency of bands was as follows: 4, 32%; 3, 31%; 2, 19%; and 1, 18%. Band 2 and the EIA test detected antibodies to the same c100-3 fragment and showed 74% concordance. No explanation is apparent for the lower concordance rate here than that between the EIA test and bands 3 = 96% or 4 = 88%. The EIA and RIBA II tests, together with positive liver function tests and abnormal tissue pathologic findings, provide a basis for the diagnosis of HCV.

Keywords

Hepatitis C; Non-A,non-B hepatitis; Anti-HCV; RIBA II band patterns

This study was designed to compare the results of EIA (c100-3 antigen) and RIBA II (four hepatitis C virus [HCV] antigens) tests for antibodies to recombinant proteins derived from the HCV genome. The recombinant antigens used in these tests were developed by Chiron Corporation (Emeryville, CA)^{1,2} and the test kits distributed by Ortho Diagnostics, Inc. (Raritan, NJ). Because the EIA test has been approved by the United States Food and Drug Administration, we used it to declare a sample anti-HCV + or 0. The new test, herein called RIBA II, is a Chiron RIBA second generation assay using five recombinant antigens,

including c100-3. Other antigens are 5-1-1, c33c, c22-3 (a structural antigen), and SOD (a non-viral antigen). This second-generation test is not Food and Drug Administration approved and can be used only for research. Thus results obtained in this laboratory cannot be linked to patient identities, used for diagnosis, or reported to primary caregivers.

The EIA and RIBA II tests were performed on a group of 1,182 “high-risk” patient samples residual from coagulation studies done at or before liver transplant. These patients were considered “high risk” for anti-HCV because many had received multiple transfusions before blood donations were tested routinely for anti-HCV (May 1990). All patients had life-threatening liver disease. Infection with HCV, if it had occurred, may have been primary or coincidental.

MATERIALS AND METHODS

The plasma samples were left over from coagulation studies and had been stored at -70°C for periods of up to 8 years. Usually they were baseline samples from primary liver transplants but if these were exhausted, the second sample taken just before the anhepatic phase of the operation was used. This research was considered exempt from the need for written consent and was approved by the University of Pittsburgh’s Institutional Review Board.

The EIA test for anti-HCV was “Hepatitis C virus Encoded Antigen (Recombinant c100-3) Ortho HCV ELISA Test System.” The manufacturer’s suggested procedure was followed. Initially reactive specimens were retested in duplicate and considered repeatedly reactive (+) if either or both duplicates were greater than the cutoff value. The EIA results were reported as + or 0.

The EIA optical density readings were scored as follows: $< \text{cutoff} = 0$, $\text{cutoff to } 2 \times \text{cutoff} = 1$, $> 2 \times \text{cutoff} = 2$. Each initial reaction (1 or 2) was repeated twice. All three readings were added together for the score. That is, EIA = 0 or 1/00 or 2/00 and EIA = + or 1/01 (2), 1/11 or 2/10 (3), 1/21 or 2/11 (4), 2/12 or 1/22 (5) and 2/22 (6).

The RIBA II test was “Chiron RIBA HCV Test System Second Generation Assay,” distributed by Ortho Diagnostic Systems, Inc. The manufacturer’s suggested procedures were followed. Results were reported as P (positive) if two or more bands were found, I (indeterminate) if only one band resulted, and N (negative) if no band formed. In the final analysis of band patterns, intensities of $\pm$ (less than control I) were considered negative. Otherwise, band intensities were not used. Band patterns were described by the location of stained bands (position 1, 2, 3, or 4). There were no reactions to the nonviral band SOD, human superoxide dismutase. This material is derived from the recombinant DNA technology used to produce the antigens that are all SOD fusion proteins. It is included on the cellulose strip to detect an antibody to this nonviral portion of the HCV recombinant antigens.

We used chi-square analysis for statistical evaluation.

RESULTS

Concordance Rate

Hepatitis C virus markers are reported with the EIA (E) results first, 0 or +, and the RIBA II (R) results second, N, P or I. Thus 0N indicates negative and + P positive results in both tests. Table 1 shows the concordant and discordant results. If the indeterminate readings in

which only one band was visualized are included with the Ps, 91% are matches, 0N or + P/I, and the concordance rate between the two tests is highly significant at $P < 0.0005$.

EIA Optical Density Score

The actual optical density (OD) readings were available on 799 of the 1,182 patient sample tests. Table 2 shows the OD score compared to the number of RIBA II bands. A sample with high EIA score most frequently showed multiple bands. Actually, with EIA + (OD 6, 5, 4, 3, 2), there were 131 with band(s) and 8 without bands. When EIA was 0 (score = $2 \times$ IR only positive) there was only one with bands. The EIA ODs less than the cutoff value were associated with no bands in 653 tests.

Band Patterns in RLBA P or I Samples

By definition, RIBA-positive (P) samples produce two to four visible antibody bands to the individual HCV recombinant antigens and indeterminate (I) samples respond with one band. The observed band positions are shown in Table 3. There are 11 possible positive patterns:

Four antibodies—one pattern = 1234

Three antibodies—four patterns = --234, 1-34, 12-4, and 123-

Two antibodies—six patterns = 12--, 1-3-, 1--4, -23-, -2-4, and --34

Indeterminate results show only one band, thus only four patterns are possible, 1 or 2 or 3 or 4.

Table 2 shows that when EIA and RIBA II results were both positive (+P), pattern 1234 was most frequent, appearing in 47% of patient samples. The two-band pattern, 34, was next most frequent, found in 16%. When the EIA test result was negative (0P), almost the reverse was found. Pattern 1234 appeared in none and the pattern 34 occurred in 87% of patient samples. This is illustrated in Figure 1.

Band Frequency

In all RIBA II-positive (+ P, 0P) and RIBA II-indeterminate (+I, 0I) samples, band 4 occurred 191 times (32%), band 3 occurred 183 times (31%), band 2 occurred 110 times (19%), and band 1 occurred 104 times (18%).

Band 2 and EIA Test Concordance

The EIA test uses the c100-3 antigen, as does the RIBA II test's band 2, thus concordance between EIA positivity and band 2 visualization is expected. Table 4 shows that concordance occurred in 168 samples (74%). This was significant at $P < 0.005$. Using similar comparisons, concordance was greater with bands 3 (96%) and 4 (88%).

ER Categories in Various Liver Disorders

Table 5 lists the numbers of patients falling into each liver diagnosis and the number and percentage within each diagnosis with different ER categories. The discordant result + N was found most frequently in autoimmune hepatitis (12%), chronic hepatitis B (11%), and acute hepatitis (10%). The result OP/I occurred most commonly in non-A,non-B hepatitis (13%).

Discussion

The studies of Alter and associates³ suggest that hepatitis C is the major if not the only cause of non-A,non-B hepatitis. It is desirable to have an accurate method of detecting the virus

itself or an immunologic response to the virus early in the course of the disease. This will help to identify individuals who are at risk of transmitting the disease or in whom the disease may progress. The two tests reported here were in good agreement when results were negative (0N) or positive (+ P/I) in both. The concordance rate was high and statistically significant. The discordant values are either + N or OP/I. The ER pattern + N is thought to be an EIA “false-positive” result perhaps due to hypergammaglobulinemia.⁴ It is also possible that it is an early manifestation of HCV in which the antibody has insufficient affinity to react in the RIBA II test. On the other hand, the discordant OP/I may represent a stage in the development of a full-positive test result that is found in the window between HCV exposure and EIA positivity. Current second-generation EIA assays support this theory.⁵ Our studies do not offer evidence to support either of these premises. The precise date of exposure to the virus was unknown and serial preoperative samples were unavailable for analysis.

Of note is the finding that 37% of those patients designated as having non-A,non-B hepatitis hepatitis tested negative for both tests. Positivity in these tests clearly means that the patients have been infected with HCV but it does not tell us whether they are now immune or potentially infectious. Further study will be required to differentiate these states. Food and Drug Administration approval of the RIBA II system will allow its use as a confirmatory test in all EIA-reactive blood donors and patients. In addition, information regarding the stage of the immunologic response may be obtained through analysis of band patterns. If suspicion of infection is very strong, the RIBA test may prove useful in circumventing a false-negative EIA result. All EIA-reactive individuals are ineligible as blood donors. It remains to be determined if this policy is desirable for cadaveric organ donors with a previous history of good health, current normal liver function tests, and ready availability of tissues for histopathologic examination.

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FIG. 1.
Band pattern distribution in +P and 0P samples.

TABLE 1

ER (EIA-RIBA II) of Baseline Plasma Samples from 1,182 Patients Undergoing Primary Liver Transplant

Concordant		Discordant	
ER	Number	ER	Number
0N	912	+N	44
+P	149	0P	31
+I	15	0I	31
Total	1076 (91%)		106 (9%)

ER = EIA-RIBA II pattern.

TABLE 2

Comparison of EIA Optical Density Score and RIBA II Results in 799 Patients on whom EIA Optical Density were available

		Number of Bands/RIBA II Readings					Total
		4	3	2	1	0	
EIA +							
OD score	P	P	P	P	I	N	
6	53	30	21	9	3	116	
5	0	1	3	0	1	5	
4	2	1	1	2	1	7	
3	1	1	1	3	2	8	
2	0	1	1	0	1	3	
EIA 0							
OD score							
2	0	0	0	0	4	4	
1	0	0	1	0	1	2	
0	0	0	0	0	653	654	
							799

EIA = enzyme immunoassay; OD =optical density.

TABLE 3

RIBA II Band Patterns in EIA Positive and Negative Plasma Samples (Band 1 = 5-1-1; Band 2 = C100-3; Band 3 = C33C; Band 4 = C22-3)

EIA	RIBA	Total	Bands Present in RIBA-Positive Samples											
			1234	--234	1-34	12-4	123-	12--	1-3-	1-4	-23-	-2-4	--34	
+	P	149	72	19	13	1	11	2	2	2	2	1	24	
	%		48	13	9	1	7	1	1	1	1	1	16	
0	P	31	0	2	1	0	0	0	0	1	0	0	27	
	%		0	6	3	0	0	0	0	3	0	0	87	
			<i>Bands Present in RIBA-Indeterminate Samples</i>											
			1				2		3				4	
+	I			15		1		1		4			9	
	%					6		6		27			60	
0	I			31		0		1		9			21	
	%					0		3		29			68	

EIA = enzyme immunoassay.

TABLE 4
EIA Positivity Compared to Band 2 Visualization in 226 RIBA II Positive (P or I) Samples

Concordant		Discordant	
EIA	Band 2	No.	No.
+	+	109	0
0	0	59	3
		168 (74%)	58 (26%)

EIA = enzyme immunoassay.

TABLE 5
ER (EIA/RIBA II) Categories in Patients Listed by Frequency of Pathologic Diagnoses

Liver Diagnosis	Number of Patients	Number and (%) in each ER Category					
		0N	+P	+I	+N	OP	OI
Cryptogenic cirrhosis	205	149 (73)	36 (18)	3 (1)	5 (2)	7 (3)	5 (2)
Primary biliary cirrhosis	178	167 (94)	4 (2)	0 (0)	5 (3)	0 (0)	2 (2)
Sclerosing cholangitis	112	100 (89)	6 (5)	1 (1)	2 (2)	2 (2)	1 (1)
Malignancy	110	93 (85)	10 (9)	1 (1)	0 (0)	4 (4)	2 (2)
Alcoholic cirrhosis	103	82 (80)	10 (10)	1 (1)	2 (2)	4 (4)	4 (4)
Cirrhosis—uncertain diagnosis	102	74 (73)	15 (15)	0 (0)	4 (4)	2 (2)	7 (7)
Chronic hepatitis B	95	63 (66)	14 (15)	4 (4)	11 (12)	0 (0)	3 (3)
Chronic NANB hepatitis	86	32 (37)	37 (43)	2 (2)	4 (5)	7 (8)	4 (5)
Acute hepatitis	49	38 (78)	3 (6)	2 (4)	5 (10)	0 (0)	1 (2)
α_1 Antitrypsin deficiency	29	23 (79)	5 (17)	0 (0)	1 (3)	0 (0)	0 (0)
Budd-Chiari	20	19 (95)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)
Autoimmune hepatitis	17	13 (76)	2 (12)	0 (0)	2 (12)	0 (0)	0 (0)
Wilson's disease	16	14 (88)	1 (6)	0 (0)	1 (6)	0 (0)	0 (0)
Miscellaneous*	60	51 (85)	4 (7)	1 (2)	2 (3)	0 (0)	2 (3)
Total	1182	918 (78)	148 (12)	15 (1)	44 (4)	26 (3)	31 (3)

* The miscellaneous group of 60 included: secondary biliary cirrhosis (14), hemochromatosis (9), pediatric cirrhosis (6), Caroli's disease (6), submassive hepatic necrosis (4), hemangioma (4), biliary atresia (3), congenital hepatic fibrosis (3), intrahepatic venoocclusive disease (3), cystic fibrosis (2), trauma to liver (2), chronic hepatitis (nonviral) (1), Byler's disease (1), polycystic liver disease (1), and poisoning (1).

EIA = enzyme immunoassay; NANB = non A, non B.