Hepatitis C Testing: Comparison of Ortho's EIA and RIBA II Tests in 1183 Patients Undergoing Primary Liver Transplantation

MAYA ROCHLANI, M.D., JESSICA H. LEWIS, M.D., GLENN E. RAMSEY, M.D., FRANKLIN A. BONTEMPO, M.D., GUNJAN SHAH, M.D., REBECCA A. BOWMAN, R.N., DAVID H. VAN THIEL, M.D., AND THOMAS E. STARZL, M.D., PH.D.

Central Blood Bank, Pittsburgh, Pennsylvania and the Departments of Medicine, Pathology, and Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania.

Supported by The Blood Science Foundation, Pittsburgh, Pennsylvania

Address reprint requests to Dr. Lewis: Central Blood Bank, 812 Fifth Avenue, Pittsburgh, Pennsylvania 15219

Brief Title: Hepatitis C Testing

Plasma samples from 1183 patients undergoing primary liver transplantation were tested for anti-HCV by two methods: Ortho® HCV ELISA Test System (EIA) and Chiron® RIBA™ HCV Test System (RIBA II). 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 1075 (*91%*), discordant results in 108 (*9%*), p = <0.0005. Band patterns were described by stating the band position (1, 2, 3, or 4) and inserting a dash (-) if no band was visualized. Of +P samples, *47%* showed all four bands, pattern = 1234, and *15%* the two band pattern --34. When the EIA was negative, 0P, a reverse was seen: *8%* showed 1234 and *81%* --34. There were 226 samples which formed bands (+P 144, 0P 36, +I 15, 0I 31). The frequency of bands was 4 = *32%*, 3 = *31%*, 2 = *19%*, and 1 = *18%*. Band 2 and the EIA test detect antibodies to the same c100-3 fragment and showed *76%* concordance, p = <0.05. 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 liver function tests and tissue pathology provide a basis for the categorical diagnosis of Hepatitis C.

Key words: Hepatitis C, Hepatitis NANB, anti HCV, RIBA II band patterns.

This study was designed to compare the results of EIA (c100-3 antigen) and RIBA II (four HCV antigens) tests for antibodies to recombinant fragments of the hepatitis C virus genome (HCV). The antigens used in these tests were developed by Chiron Corporation[1,2] and the test kits distributed by Ortho Diagnostics, Inc. Because the EIA test has been approved by the FDA we used it to declare a sample anti HCV + or 0. The new test herein called RIBA II is Chiron® RIBA™ second generation assay utilizing five recombinant antigens, including c100-3. Other antigens are 5-1-1 c33c, c22-3, and SOD. This second generation test is not FDA approved and can be used only for research. Thus, results cannot be linked to patient identities, used for diagnosis or reported to primary care-givers.

EIA and RIBA II tests were done on a group of 1183 "high risk" patient samples residual from coagulation studies. These patients were considered "high risk" for anti HCV because many had received multiple transfusions before blood donations were routinely tested 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 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 exactly. Initially reactive specimens were retested in duplicate and considered positive if either or both duplicates were greater than the cut off value. EIA results were reported as + or 0.

The RIBA II test was "Chiron® RIBA™ HCV Test System Second Generation Assay." Ortho Diagnostic Systems, Inc. distributes these test kits. We are grateful for their advice and provision of some of the kits used. 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 ± (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 non-viral band SOD, human superoxide dismutase. This material is derived from the recombinant DNA technology used to produce antigens c100-3 and c22-3 in yeast. It is included on the cellulose strip to detect an antibody to this possible contaminant.

as illustrated in Fig

RESULTS

Concordance Rate

HCV markers are reported with the EIA (E) results first, 0 or +, and the RIBA II (R) results second, N, P, or I. Hence 0N indicates negative and +P positive 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 $p < 0.0005$.

Band Patterns in RIBA P or I Samples

By definition RIBA positive (P) samples produce 2 to 4 visible antibody bands to the HCV fragments and indeterminate (I) samples respond with one band. The observed band positions are shown in Table 2. There are 11 possible positive patterns:

4 antibodies - one pattern = 1234

3 antibodies - four patterns = -234, 1-34, 12-4, 123-

2 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 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 was negative (0P) almost the reverse was found. Pattern 1234 appeared in only *8%* and the pattern --34 occurred in *81%* of patient samples. This is illustrated in Figure 1.

Band Frequency

In all RIBA II positive (+P, 0P) and indeterminate (+I, 0I) samples band 4 occurred 194 times (*32%*), band 3 187 times (*31%*) band 2 112 times (*19%*) and band 1 107 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 3 shows that concordance occurred in 163 samples (*76%*). This was significant at $p = <0.05$.

ER Categories in Various Liver Disorders

Table 4 lists the numbers of patients falling into each liver diagnosis and the number and percent within each diagnosis with the different ER categories. The discordant result +N was found most frequently in Autoimmune Hepatitis (12%), Chronic Hepatitis N (11%) and Acute Hepatitis (10%). OP/I occurred most commonly in NANB Hepatitis, (15%) and Autoimmune Hepatitis (12%).

DISCUSSION

The studies of Alter and his associates[3] suggest that hepatitis C is the major if not the only cause of non-A non-B (NANB) hepatitis. Thus it is desirable to have an accurate test which detects an immunologic response to the virus early in the course of the disease. The concordance or agreement between the two tests used in our studies is great and highly significant. The discordant values are either +N or 0P/I. The ER pattern +N is thought to be a "false" positive perhaps due to hypergammaglobulinemia. It is also possible to assume that it is an early manifestation of HCV in which the antibody has insufficient affinity to visualize the band. On the other hand, the discordant 0P/I may represent a stage in the development of a full positive test and might be expected to be found in the window between HCV exposure and EIA positivity. Our studies do not offer evidence to support this premise. Date of exposure to the virus is unknown and serial pre-operative samples are unavailable.

At this time we can only suggest that all blood donors and patients suspected of HCV infection be tested with an approved test. Those which are positive, especially those with abnormal liver function tests should have additional blood samples stored for future assays. The RIBA II test will be confirmatory but also, through the band patterns, may offer additional information about the stage of the immunological response.

Much further study will be required to distinguish which patients of those with HCV antibodies are potentially infectious and which are immune. Although HCV positive patients presently are disqualified as blood donors, it remains to be determined if this policy is warranted for cadaveric organ donors with a previous history of good health, normal liver function tests, and an unlimited availability of liver and other tissues for direct histopathologic examination.

References

1. Choo QL, Kuo G,Wiener AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359-362.

2. Kuo G, Choo QL, Alter HJ et al. An assay for circulating antibodies to a major etiologic virus of non-A, non-B hepatitis. Science 1989; 244:362-364.

3. Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghtom M, Choo QL, Kuo G. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med 1989; 321:1494-1500.

Acknowledgement. The authors thank Lisa Garbin for her technical assistance, Gertrude Maxon for manuscript preparation and Sami Awad for computer assistance.

TABLE 1. ER (EIA-RIBA II) OF BASELINE PLASMA SAMPLES FROM 1183 PATIENTS UNDERGOING PRIMARY LIVER TRANSPLANT

Concordant		*Discordant*	
ER	*Number*	*ER*	*Number*
0N	916	+N	41
+P	144	0P	36
+I	15	0I	31
TOTAL	1075 (*91%*)		108 (*9%*)

TABLE 2. 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)

Bands Present in RIBA Positive Samples

EIA	*RIBA*	*TOTAL*	*1234*	*-234*	*1-34*	*12-4*	*123-*	*12--*	*1-3-*	*1--4*	*-23-*	*-2-4*	*--34*
+	P	144	68	19	13	1	12	2	2	2	2	1	22
		%	*47*	*13*	*9*	*1*	*8*	*1*	*1*	*1*	*1*	*1*	*15*
0	P	36	3	2	1	0	0	0	0	1	0	0	29
		%	*8*	*6*	*3*					*3*			*81*

Bands Present in RIBA Indeterminate Samples

EIA	*RIBA*	*TOTAL*	*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*

TABLE 3. EIA POSITIVITY COMPARED TO BAND 2 VISUALIZATION IN 180 RIBA II POSITIVE SAMPLES

Concordant			*Discordant*		
EIA	*Band 2*	*No.*	*EIA*	*Band 2*	*No.*
+	+	105	+	0	39
0	0	31	0	+	4
		136 (*76%*)			44 (*24%*)

TABLE 4. ER (EIA/RIBA II) CATEGORIES IN PATIENTS LISTED BY FREQUENCY OF PATHOLOGICAL DIAGNOSES

	Liver Diagnosis	Number of Patients	Number and *(%)* in each ER Category					
			0N	+P	+I	+N	0P	0I
CC	Cryptogenic Cirrhosis	205	149*(73)*	35*(17)*	3*(1)*	5*(2)*	8*(4)*	5*(2)*
PBC	Primary Biliary Cirrhosis	178	167*(94)*	4*(2)*	0*(0)*	5*(3)*	0*(0)*	2*(2)*
PSC	Sclerosing Cholangitis	113	101*(89)*	6*(5)*	1*(1)*	2*(2)*	2*(2)*	1*(1)*
MA	Malignancy	110	93*(85)*	10*(9)*	1*(1)*	0*(0)*	4*(4)*	2*(2)*
ALC	Alcoholic Cirrhosis	103	84*(82)*	10*(10)*	1*(1)*	2*(2)*	2*(2)*	4*(4)*
CU	Cirrhosis - Uncertain Dx	102	74*(73)*	15*(15)*	0*(0)*	4*(4)*	2*(2)*	7*(7)*
CHB	Chronic Hepatitis B	95	61*(64)*	14*(15)*	4*(4)*	10*(11)*	11*(3)*	3*(3)*
NANB	Chronic NANB Hepatitis	86	34*(40)*	35*(41)*	2*(2)*	2*(2)*	9*(10)*	4*(5)*
AcHep	Acute Hepatitis	49	38*(78)*	2*(4)*	2*(4)*	5*(10)*	1*(2)*	1*(2)*
α_1ATD	α_1 Antitrypsin Deficiency	29	23 *(79)*	5*(17)*	0*(0)*	1*(3)*	0*(0)*	0*(0)*
B-C	Budd-Chiari	20	19*(95)*	1*(5)*	0*(0)*	0*(0)*	0*(0)*	0*(0)*
AuH	Autoimmune Hepatitis	17	11*(65)*	2*(12)*	0*(0)*	2*(12)*	2*(12)*	0*(0)*
WD	Wilson's Disease	16	14*(88)*	1*(6)*	0*(0)*	1*(6)*	0*(0)*	0*(0)*
MISC	Miscellaneous*	60	51*(85)*	4*(7)*	1*(2)*	2*(3)*	0*(0)*	2*(3)*
	TOTAL	1183	919*(78)*	144*(12)*	15*(1)*	41*(3)*	33*(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), Intra-Hepatic Veno-Occlusive Disease (3), Cystic Fibrosis (2), Trauma to Liver (2), Chronic Hepatitis (non-viral) (1), Byler's Disease (1), Polycystic Liver Disease (1), and Poisoning (1).

Figure 1. Band Pattern Distribution
in +P and 0P Samples