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Low-Positive Anti-Hepatitis C Virus Enzyme Immunoassay Results: An Important Predictor of Low Likelihood of Hepatitis C Infection

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Background: **Tests for hepatitis C antibodies (anti-HCV enzyme immunoassays) are usually described as positive or negative. Several studies, mainly in blood donors, have found that specimens with low signal/cutoff (S/C) ratios are commonly negative when tested with a recombinant immunoblot assay (RIBA) or for HCV RNA.**

Methods: **We retrospectively reviewed 17 418 consecutive anti-HCV results from a screening program for high-risk veterans; 2986 (17.1%) samples were anti-HCV-positive, and 490 (16.4%) had S/C ratios ≤3.7 (low positive). Additional tests were performed in 1814 anti-HCV-positive individuals.**

Results: **RIBA was performed in 263 patients with low-positive anti-HCV; results were negative in 86%, indeterminate in 12%, and positive in 2%. Only 16 of 140 individuals (11%) with low-positive anti-HCV values were HCV RNA-positive, whereas HCV RNA was positive in 90% of 1435 individuals with high-positive anti-HCV values (*P* <0.0001). Compared with those with high-positive anti-HCV, individuals with low-positive anti-HCV values were older (*P* <0.0001) and were less likely to have risk factors for HCV (*P* <0.0001 for most), multiple increased alanine aminotransferase (ALT) activity values (30% vs 81%; *P* <0.0001), or positive anti-hepatitis B core antigen (19% vs 59%; *P* <0.0002). Among 634 individuals with high anti-HCV titers and multiple increased ALT activity values, 95% were HCV RNA-positive.**

Conclusions: **The S/C ratio is important even in high-risk individuals; laboratories should report the S/C ratio along with anti-HCV EIA results and perform supplemental RIBA testing in those with low-positive values to avoid reporting false-positive results.**

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Testing for hepatitis C virus (HCV)[6] infection typically begins with measurement of antibodies to HCV proteins using enzyme-linked immunosorbent assays, more simply termed enzyme immunoassays (EIAs). Currently, laboratories use either "second-" or "third-generation" EIA tests that detect antibodies to one or more of several recombinant or synthetic proteins produced by genes from different areas of the HCV genome. In a large epidemiologic study, ~75% of those with repeatedly positive anti-HCV EIA results were found to have circulating HCV RNA *(1)*.

Anti-HCV EIA results are interpreted by comparison of absorbance readings with a defined cutoff value. Although EIA tests provide a quantitative absorbance result [often reported as the signal to cutoff (S/C) ratio], they are usually reported simply as positive or negative. Several studies using first- and second-generation anti-HCV EIA tests have shown that samples with absorbance values just slightly above the cutoff value have a significantly greater likelihood of representing false-positive results compared with those with higher values *(2–17)*.

Because of the possibility of false-positive results, especially in low-prevalence settings such as testing of

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[6] Nonstandard abbreviations: HCV, hepatitis C virus; EIA, enzyme immunoassay; S/C, signal/cutoff; RIBA, recombinant immunoblot assay; ALT, alanine aminotransferase; and HBc, hepatitis B core antigen.

blood donors, positive anti-HCV EIA results are usually confirmed by additional tests. Recombinant immunoblot assays and strip immunoblot assays (often collectively referred to as RIBA) use the same proteins as EIA tests of the same generation, but the antigens are separately localized to determine the number and identity of antigens to which anti-HCV antibodies are directed. The current clinical practice after identifying a positive anti-HCV EIA result is to measure HCV RNA to assess whether viremia is present. Several review articles have suggested that RIBA has no place in the evaluation of positive anti-HCV EIA results in the clinical setting *(18–20)*.

Before the introduction of anti-HCV EIA tests, surrogate markers of HCV infection were used to identify blood donors at high risk of transmitting hepatitis to transfused recipients of their blood. The two most helpful markers were alanine aminotransferase (ALT) activity and antibody to the hepatitis B virus core antigen (anti-HBc) *(21–24)*. Several studies have shown that there are differences in the frequency of increased ALT activity and positive anti-HBc between blood donors with low and high absorbance values for anti-HCV *(4, 9, 14, 17)*.

Despite the potential utility of anti-HCV EIA S/C ratio values and surrogate markers in evaluating test results, few studies have evaluated their utility in the setting of diagnostic testing of populations at high risk for HCV infection *(3, 8, 15)*. Individuals seeking care in Veterans Affairs Medical Centers have been recognized as a population at high risk of HCV infection. In a random, nationwide single-day study of prevalence of anti-HCV among veterans seeking care, 6.6% were positive; in many urban medical centers, the prevalence was >10% *(25)*. In a preliminary study among veterans, we reported a low frequency of positive RIBA results for patients with low absorbance values, using a second-generation anti-HCV EIA test *(26)*. We here extend these observations to a large number of patient samples tested by third-generation anti-HCV EIA assays. We also evaluated the utility of surrogate markers in individuals with positive anti-HCV EIA results.

Materials and Methods

The Washington, DC Veterans Affairs Medical Center performed all hepatitis serology testing for the three hospitals in Veterans Integrated Service Network 5 from July 2000 through March 2002. The Department of Veterans Affairs has an active program of surveillance for hepatitis C and has introduced a hepatitis C risk factor questionnaire for evaluation of all primary care patients who do not already have a positive anti-HCV result. The questionnaire includes inquiries about lifetime abuse of illicit drugs or ethanol, transfusions before 1992, multiple sex partners, tattoos or multiple piercing, needlesticks or other exposure to blood, and service in Vietnam. Substance abuse was further characterized as intravenous, nasal, or smoking, although this information was not available for all patients. Among those with a history of substance abuse, information on injection drug use was available for 84%; of these, 85% admitted to injection drug use and 15% admitted only to snorting or smoking drugs. Ethanol abuse was defined as more than three drinks per day on a recurring basis or a positive CAGE questionnaire. Individuals who indicate the presence of any risk factors for HCV infection are offered hepatitis C screening by EIA tests. Testing is also offered to all individuals who request it, even if they deny risk factors. Hepatitis C screening was performed on a Labotech (Adaltis US Inc.) using a third-generation anti-HCV EIA assay (Ortho Clinical Diagnostics). Analyses were performed according to the manufacturer's specifications; a result was considered positive when it was >0.6 absorbance units above the mean of the negative control values (termed the cutoff point). Results from individual patient samples are reported using the S/C ratio; specimens with a S/C ratio >1 are tested in a second run and reported as positive only if both values are above the cutoff value ("repeatedly reactive"). The CV of the S/C ratio was 9.2% at a ratio of 3.0.

Before October 2000, decisions on whether to perform additional tests were made by the physician caring for the patient in one hospital. From October 24, 2000, through the end of the study, all but 1 of 210 specimens at this hospital with positive anti-HCV EIA results but with a S/C ratio <3.5 were retested using a third-generation RIBA (Chiron Corporation); there was insufficient sample remaining to perform a RIBA in the remaining case. RIBA was performed, when requested by physicians, on an additional 54 samples with low-positive anti-HCV results. From July 2001 through the end of the study, a sample was collected for HCV RNA by in-house PCR (lower detection limit, 10^2–10^3 copies/mL) on all patients at a second hospital and run automatically if the anti-HCV result was positive (regardless of S/C ratio). Measurement of HCV RNA at the other two hospitals was performed, if ordered by the physician, with a commercial qualitative PCR method (Amplicor; Roche Molecular Systems; lower detection limit, 50 IU/mL) or quantitative branched DNA method (Bayer Diagnostics; lower detection limit, 615 IU/mL). The percentage of samples positive by each of the three methods was similar (90–92%).

We retrospectively reviewed results from a blinded database of HCV EIA S/C ratios of all patients tested over a 21-month period. In all samples with positive anti-HCV results, we reviewed supplemental test results (RIBA and HCV RNA), risk factors for HCV infection, ALT activity measurements, and hepatitis B serology results. The study design was approved by the Medical Center Institutional Review Board. Differences between groups were evaluated using the χ^2 test; results were considered significant at $P < 0.05$. Differences in age and in S/C ratio were evaluated by the *t*-test for samples with unequal variances.

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Results

Over a 21-month period, we tested 17 418 specimens from individuals with no previous positive anti-HCV result; 2986 specimens (17.1%) were repeatedly reactive. These individuals were predominantly male (96%) with a mean (SD) age of 52.3 (10.2) years; 66% were of African-American, 33% of European, and 1% of Hispanic ancestry. The distribution of S/C ratios is illustrated in Fig. 1. The mean (SD) S/C ratio was 4.5 (1.3). Because of the biphasic nature of the distribution, we selected a S/C ratio of 3.5 as the apparent break point between the two curves, excluded values lower than the break point, and calculated the mean S/C ratio of the main distribution as 5.0 (0.4). Including values within 3 SD of the mean gave a lower cutoff value of 3.8 for positive results. A total of 490 samples had a S/C ratio ≤3.7 (16.4% of all positive results) and comprised the low-positive group. Individuals with low-positive anti-HCV values were significantly older than those with high-positive anti-HCV values [57.9 (13.5) years vs 50.3 (7.3) years; $P < 0.0001$]. Although 66% of those with high-positive anti-HCV values were between the ages of 45 and 54, only 27% of those with low-positive anti-HCV values fell within this age range. In addition, although only 6.5% of those with high-positive anti-HCV values were over age 65, 34% of those with low anti-HCV titers were 65 or older (Fig. 2).

Influenza vaccinations may cause false-positive results for anti-HCV and other serologic tests *(27)*, which might cause an increase in low-positive anti-HCV results in the fall. To determine whether the distribution of low-positive results varied by time of year, we separately analyzed the frequency of low-positive results by month collectively and for each facility separately. There was no consistent temporal pattern in the three hospitals (data not shown). The frequency of low-positive results was 12% before the screening instrument was introduced but increased to 20% after its introduction ($P < 0.001$).

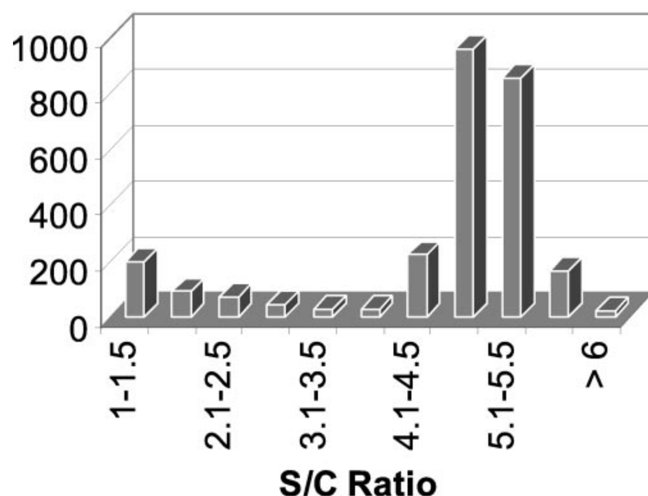

Fig. 1. Distribution of anti-HCV S/C ratio.

In 2986 samples positive for anti-HCV by a third-generation EIA assay, the S/C ratios followed a biphasic distribution. The natural break point of the distribution occurred as a S/Catio of 3.7 or lower for low-positive results.

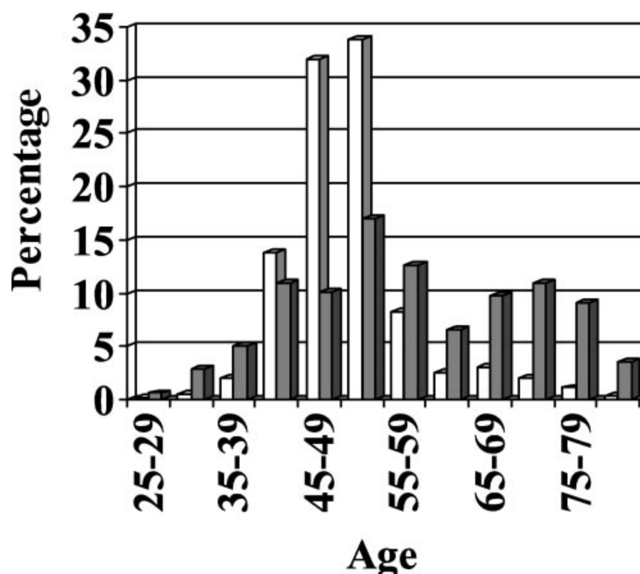

Fig. 2. Age distribution of anti-HCV results.

Individuals with high-positive anti-HCV (□) were predominantly middle-aged adults, with 88% between 40 and 60 and 66% between 45 and 55 years of age. In contrast, individuals with low-positive anti-HCV (▨) had a more uniform age distribution; only 51% were between 40 and 60 years of age, whereas 34% were over age 65.

Although individuals were offered screening based on high risk of HCV infection, individuals with low-positive anti-HCV results were significantly less likely than those with high-positive results to have recognized risk factors for HCV infection (with the exception of dialysis, which was significantly more common in those with low-positive anti-HCV results); the data on individual risk factors are summarized in Table 1. More than one-half of individuals with low-positive anti-HCV results had no recognized risk factors for HCV infection. In contrast, only 11% of those with high anti-HCV titers and positive HCV RNA results lacked recognized risk factors ($P < 0.0001$). Those

Table 1. Comparison of risk factors in persons with high and low anti-HCV results.

Risk factor[a]	High-positive, %	Low-positive, %	*P*
No information available	5.9	7.5	NS[b]
No risk factor identified	11.2	55.7	<0.0001
Substance abuse	78.2	17.4	<0.0001
Transfusion	29.2	20.5	0.034
Multiple sex partners	47.0	16.8	0.0001
Needlestick	3.4	5.4	NS
Dialysis	0.9	3.3	0.008

[a] Percentages reflect number of persons admitting to risk factor compared with number of persons for whom information on the risk factor was available. Many individuals admitted to more than one risk factor. No risk factor identified was coded only in persons who denied all risk factors listed.

[b] NS, not significant.

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Table 2. HCV RIBA results at different anti-HCV EIA reactivity values ≤3.7.

Anti-HCV EIA	Positive	Indeterminate	Negative
1.0–1.5 (n = 125)	1 (1%)	10 (8%)	114 (91%)
1.6–2.0 (n = 43)	1 (2%)	7 (16%)	35 (82%)
2.1–2.5 (n = 51)	1 (2%)	5 (10%)	45 (88%)
2.6–3.0 (n = 27)	1 (4%)	5 (19%)	21 (77%)
3.1–3.5 (n = 16)	0	3 (19%)	13 (81%)
3.6–3.7 (n = 1)	0	1 (100%)	0

with low anti-HCV titers were also significantly less likely to have a history of an icteric illness (1.2% vs 10.8%; $P < 0.0002$). The frequency of injection vs noninjection drug use did not differ between the two groups.

RIBA was performed on 263 specimens with low-positive EIA values; the results are shown in Table 2. Only 4 specimens (2%) were RIBA-positive, whereas 31 (12%) were RIBA indeterminate. All of the indeterminate results had reactivity to only one HCV protein: NS5 in 12 samples, c33c in 10 samples, 5-1-1 (c100p) in 5 samples, and c22p in 4 samples. Twelve of the individuals with indeterminate RIBA (8 with NS5 and 4 with c33c) also had HCV RNA performed; results were negative in 11 and positive in 1 with antibody to c33c. The test was not performed in the remaining 19 RIBA-indeterminate individuals. Three of the positive specimens had antibodies to both c22p and c33c, whereas the other was positive for all four HCV bands; HCV RNA was negative in three and not performed in the other one.

The HCV RNA assay was performed for 1575 anti-HCV EIA-positive individuals; 1313 (83%) had detectable HCV RNA. The distribution of positive results differed markedly between those with low-positive and high-positive results, as shown in Table 3. Only 16 of 140 samples (11%) from persons with low-positive anti-HCV results were HCV RNA-positive. Twenty-three of the 263 patients tested by RIBA also had undetectable HCV RNA (included in the 140 low-positive anti-HCV samples mentioned above); RIBA results were positive in 1, indeterminate in 10, and negative in the remaining 12. In contrast, 1297 of 1435 specimens (90%) from patients with high-positive anti-HCV results had positive HCV RNA results ($P < 0.0001$). Individuals with high-titer anti-HCV but negative for HCV RNA had a mean age of 53.2 years, significantly older than those positive for HCV RNA ($P < 0.005$). HCV RNA-negative individuals were significantly more likely to lack risk factors for HCV (8.9% vs 2.8%; $P < 0.003$) and were less likely to have a history of substance abuse (58% vs 78%; $P < 0.0001$) or an acute icteric illness (2.6% vs 10.8%; $P < 0.0001$). There were no other significant differences in risk factors between the two groups.

Table 3. HCV RNA results at different anti-HCV EIA reactivities.

Anti-HCV EIA	Total	Positive	Negative
1.0–1.5	45	3 (7%)	42 (93%)
1.6–2.0	34	4 (12%)	30 (88%)
2.1–2.5	26	3 (12%)	23 (88%)
2.6–3.0	12	2 (17%)	10 (83%)
3.1–3.5	13	2 (15%)	11 (85%)
3.6–3.7	10	2 (20%)	8 (80%)
3.8–4.0	8	7 (88%)	1 (12%)
4.1–4.5	120	104 (87%)	16 (13%)
≥4.6	1307	1186 (91%)	121 (9%)

Table 4. Patterns of ALT activity in 1007 anti-HCV-positive patients with at least four ALT activity values, based on HCV RNA and RIBA status.

	High-titer anti-HCV		
ALT pattern	HCV RNA-positive	HCV RNA-negative	Low-titer anti-HCV and negative RIBA and/or HCV RNA
All within reference limits (n = 223)	77 (35%)	69 (31%)	77 (35%)
Only one increased (n = 104)	60 (58%)	26 (25%)	18 (17%)
Multiple increased (n = 680)	600 (88%)	34 (5%)	46 (7%)

A total of 1007 patients with positive anti-HCV EIA values had at least four ALT activity measurements recorded in their medical records at or before the time of anti-HCV testing. The frequency of increased ALT activities differed markedly between individuals positive for HCV RNA and those with undetectable HCV RNA or negative RIBA results, as shown in Table 4. Only 10% of individuals positive for HCV RNA had ALT continuously within the reference interval, compared with 54% of those with undetectable HCV RNA or a negative RIBA result ($P < 0.0001$). Eighty-one percent of patients positive for HCV RNA had multiple increased ALT values, a finding noted in only 26% of those with undetectable HCV RNA and in 33% with a negative RIBA results ($P < 0.0001$). Of 223 patients with ALT activity consistently within the reference interval, HCV RNA was detected in 77 (35%). In 104 patients with a single increased ALT activity measurement, HCV RNA was detected in 58%, whereas HCV RNA was present in 88% of 680 patients positive for anti-HCV and with multiple increased ALT activity values and in 95% of those with high-titer anti-HCV and multiple increased ALT activity values.

Complete hepatitis B serologies were available for 1206 patients with positive anti-HCV EIA results and known HCV RNA or RIBA status. Anti-HBc was detected in 572 of 968 (59%) patients who were HCV RNA-positive, in 45 of 71 (63%) persons with high anti-HCV titers and negative HCV RNA, but in only 31 of 167 (19%) individuals with low anti-HCV titers and undetectable HCV RNA or negative RIBA results ($P < 0.0002$ compared with high-titer, RNA-positive individuals). Of 327 persons with anti-HCV and isolated positivity for anti-HBc, 308 (94%) were HCV RNA-positive.

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To determine whether immunosuppression related to HIV infection might be related to the frequency of samples with low-positive anti-HCV results, we compared the frequency of positive HCV RNA and RIBA values in patients who were HIV-positive or -negative. Among 125 patients who were HIV-positive, the frequency of low-positive results was 15.6%, similar to than that seen in HIV-negative individuals. The mean S/C ratio in HIV-positive individuals (5.0 ± 1.3) was not significantly different from that in HIV-negative persons (5.0 ± 1.0; $P = 0.91$). In contrast, 5 of the 16 samples positive for HCV RNA from patients with low-positive anti-HCV values were HIV-positive.

Discussion

Our results confirm those of previous studies, performed primarily in blood donors, indicating that low-positive anti-HCV EIA results frequently represent false-positive reactivity. Other studies of anti-HCV EIA assays also noted that individuals with low-positive results were typically negative when tested by RIBA or HCV RNA *(28, 29)*. Package inserts from manufacturers indicate this fact but do not suggest reporting results along with S/C ratio or supplementary testing in individuals with low-positive anti-HCV EIA results. In our study of almost 3000 anti-HCV-positive specimens, ~1 in 6 positive results was low-positive. Individuals with low-positive anti-HCV results were significantly older than those with high-positive anti-HCV results, and the majority lacked recognized risk factors for HCV infection. Additionally, those with low-positive anti-HCV results were seldom positive for anti-HBc and usually had ALT activities within the reference interval. They appear to represent a different demographic group than do those with high-positive anti-HCV values.

Additional laboratory testing showed that almost all low-positive anti-HCV samples had negative results on supplemental testing. Most samples were negative for HCV RNA when tested: only 11% were HCV RNA-positive. Supplemental RIBA testing was also negative in the vast majority (86%) of those with low-positive anti-HCV values, and only four specimens had positive RIBA results (the three tested were negative for HCV RNA). Among the 31 samples with indeterminate RIBA results, 22 were attributable to isolated antibodies to c33c or NS5 proteins. Damen et al. *(30)* evaluated a third-generation RIBA in 530 anti-HCV-positive individuals. Only 1 of 220 HCV RNA-positive individuals had indeterminate RIBA, and none had a negative RIBA result. They found only 1 of 55 individuals with isolated antibody to c33c, and none of 51 individuals with isolated antibody to NS5 to be HCV RNA-positive. Overall, 20% of RIBA results in their study had one of these two indeterminate patterns *(30)*. In two other studies, HCV RNA was never detected in individuals with isolated antibody to NS5 on RIBA *(31, 32)*. Although the number in our study was small, we found HCV RNA in only 1 of 12 persons with isolated anti-NS5 or anti-c33c. These data support our conclusion that most low-positive anti-HCV results represent cross-reactive antibodies causing false-positive anti-HCV results.

Another possible explanation for the low-positive EIA results with negative or indeterminate RIBA results and negative HCV RNA is that these individuals had been truly HCV infected in the past, resolved the infection, and had a decrease in their titer of antibody. Several studies have shown that antibody titers fall if HCV RNA is cleared and may disappear many years after infection *(33–35)*. In the two long-term studies *(34, 35)*, 24–46% of individuals initially infected with HCV were HCV RNA-negative on follow-up, and 15–30% of these had lost anti-HCV when tested 20–30 years after initial exposure to HCV. The fact that individuals in our study with low-positive anti-HCV values were older than those with high-titer antibody could support this interpretation. This may be the explanation for low-positive results in some of our cases, as there are individuals with recognized risk factors for HCV infection. However, this is unlikely to explain the positive results for the majority of individuals with no risk factors. There are no published data to determine whether those with resolving infection lose RIBA reactivity before they lose detectable anti-HCV by EIA.

An alternative approach to evaluating individuals positive for anti-HCV is to perform HCV RNA measurements rather than RIBA; several studies have suggested this approach *(18–20)*. These recommendations are based on evaluation of persons with clinical evidence of chronic liver disease to determine the need for treatment. It is also important to distinguish individuals who have been truly exposed to the virus from those with falsely positive results for reasons of public health, avoidance of worry from labeling individuals as HCV-positive, and expense of further physician visits and laboratory testing for evaluation of false-positive results. In testing for HIV, results of EIA tests are never reported unless the Western blot (an analogous test to RIBA) is positive to rule out cross-reacting antibodies that may cause falsely positive results. We believe that this is important in testing for HCV as well. Our data, and those of other studies, have shown that the overwhelming majority of persons with low-positive anti-HCV values are negative on both RIBA and HCV RNA. The advantage of RIBA is that persons with negative RIBA results can be reported as being negative for anti-HCV, eliminating the need for further evaluation, counseling, and public health reporting. We recommend that laboratories adopt such an approach.

In published studies, the exact cutoff value used to distinguish "low positive" from "high positive" has varied, and no specific criteria to determine the cutoff value for this distinction have been proposed. We suggest two potential approaches for distinguishing low-positive from high-positive results. The first involves inspection of the distribution for a "cutpoint" dividing the two groups, whereas the second uses the mean of the major distribu-

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tion minus 3 SD; in our data, these led to a similar discriminant point of 3.8 as the lower limit of the high-positive group. Two of 13 individuals were HCV RNA-positive with S/C values between 3.1 and 3.5, 2 of 10 were HCV RNA-positive with S/C values of 3.6 or 3.7, and 7 of 8 were HCV RNA-positive with S/C values of 3.8–4.0. We thus believe that a S/C ratio ≤3.7 provides the best decision point for separating low- and high-positive anti-HCV results in our assay. At a recent consensus conference held by the CDC, the same cutoff limit of 3.8 or above for the high-positive group was suggested, based on studies in low- and high-risk populations.

Before the advent of EIA tests for anti-HCV, surrogate markers such as ALT and anti-HBc were useful in identifying persons likely to transmit non-A, non-B hepatitis *(21–24)*. Our study confirms the utility of multiple ALT activity measurements in suggesting the presence or absence of HCV RNA. The vast majority of individuals negative for HCV RNA had either ALT continuously within the reference interval or only a single increased value before the time of anti-HCV testing; HCV RNA testing in such individuals is important to distinguish viremic from nonviremic individuals. On the other hand, the positive predictive value (for viremia) of repeatedly increased ALT (in individuals with at least four ALT activity values) and high anti-HCV titers was 95% in our series. Goncales et al. *(17)* obtained positive HCV RNA results for 92% of those with high anti-HCV titers and increased ALT in a single determination. These data suggest that there is little benefit to "confirmatory testing" in those with repeatedly increased ALT activity values and high anti-HCV titers.

In several previous studies, a significant difference in the frequency of positive anti-HBc results was seen between individuals with low-positive and high-positive anti-HCV EIA results *(2, 9, 16)*. Our data confirm the results of those studies. The predictive value of isolated positive anti-HBc was 94% among those with high anti-HCV titers, similar to that for multiple increased ALT activity values. From a diagnostic standpoint, however, finding a negative anti-HBc is not strong evidence that the patient does not have HCV infection. In our study, 41% of HCV RNA-positive individuals lacked anti-HBc. Unlike ALT, anti-HBc appears unsuitable for use in classifying patients positive for anti-HCV.

There are several possible limitations to our study. The first limitation is that decisions on performing additional testing were based largely on clinical evaluation in the initial phase of our study, and not all patients with low anti-HCV titers had additional testing performed. Later, reflex testing was added; in one hospital, RIBA was performed, whereas in a second, HCV RNA was assayed. The percentage of positive HCV RNA results did not change for the group as a whole after routine reflex testing was introduced. Because no further testing was done in ~40% of patients, it is possible that some of the patients with negative RIBA results were, in fact, HCV RNA-positive. However, several studies have shown that a negative result in the third-generation RIBA is virtually never associated with positive HCV RNA results except in the setting of acute hepatitis C infection *(17, 36, 37)*.

The second limitation is that patients with increased ALT activity may have been more likely to be referred to the liver clinic and to have had the HCV RNA assay performed than patients with ALT within reference values. Conceivably, this may have led to bias in establishing the percentage of patients with high-titer anti-HCV who had positive results. In two of the hospitals, HCV RNA was assayed routinely in patients with initially positive anti-HCV EIA results. In those two institutions, 91% of those with high-titer anti-HCV and increased ALT activities also were positive for HCV RNA, whereas 98% of those referred to liver clinic in the third hospital were positive ($P < 0.0005$). Because different assays were used in the two sites doing reflex testing, we cannot rule out that differences in sensitivity between the assays was responsible for the difference.

A final potential bias of our study is the high prevalence of HCV positivity in veterans. In fact, over the 21-month period of the study, 17.1% of samples tested were anti-HCV-positive. The high prevalence of HCV in this population may have increased the positive predictive value of the results. The study of Goncales et al. *(17)*, performed in blood donors, showed a similar predictive value for high-titer anti-HCV in donors with increased ALT.

We believe that a stepwise approach is appropriate for evaluation of patients positive for anti-HCV, as shown in Fig. 3. Our data suggest that it is important for laboratories to report the S/C value whenever a positive anti-HCV result is found and for laboratories or manufacturers to determine an appropriate cutoff point to distinguish between low- and high-positive anti-HCV results. In those

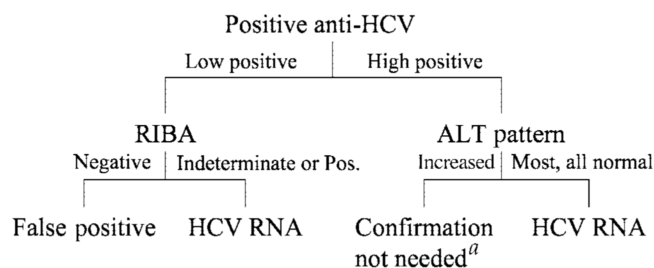

Fig. 3. Suggested approach to positive anti-HCV results.

In contrast to current evaluation schemes, our data suggest that different approaches should be used in persons with low-positive and high-positive anti-HCV results. In low-positive individuals, most results will be false positive, so that RIBA (which can identify false positive reactions) should be the initial test of choice. In contrast, in persons with high-positive anti-HCV results, RIBA is virtually always positive or indeterminate, providing no useful data. Because many individuals with low-positive anti-HCV results and indeterminate or positive RIBA, or high-positive anti-HCV with ALT within the reference limits, will be negative for HCV RNA, routine confirmation with HCV RNA measurement is helpful in assessing true HCV status. In contrast, 95% of persons with high-titer anti-HCV and multiple increased ALT values are HCV RNA-positive, suggesting that there is little need for confirmatory testing in such patients unless treatment is considered. *a*, quantitative HCV RNA assay should be performed before treatment.

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with low ratios, confirmatory testing is necessary because of the high frequency of false-positive results. We believe that RIBA testing should be performed first because a negative RIBA prevents false labeling of individuals as HCV exposed. Measurement of HCV RNA should be reserved for patients with indeterminate or positive RIBA results. In contrast, in those with high S/C ratio, HCV RNA will be positive in most patients. We believe that confirmatory testing is not needed in such individuals unless ALT is consistently within reference limits. Because the HCV RNA viral load is an important predictor of duration of therapy *(38)*, quantitative HCV RNA should be determined before anti-HCV-positive individuals are started on treatment to provide prognostic information.

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