Objectives: Hepatitis C virus (HCV) is not directly cytopathic to the hepatocytes; however, host immune response against the virus does cause hepatic injury. Production of the HCV antibody is a host immune response to a viral antigen. The currently used HCV antibody assay is a qualitative, not quantitative, assessment. In this study, we sought to quantitatively estimate HCV antibody levels in patients who had undergone liver transplantsations at the University of Rochester Medical Center, Rochester, New York, and correlate these levels with HCV RNA viral load, genotype, severity of recurrence, and anti-HCV treatment.

Materials and Methods: From 39 liver transplantation patients, we obtained 141 blood samples for quantitative HCV RNA to measure HCV antibody levels quantitatively.

Results: Most antibody levels were within a narrow range with a mean of 32.9 ± 5.1. Samples with undetectable RNA had a mean antibody level of 31.4 ± 8.0, and samples with a positive RNA had mean level of 33.0 ± 4.6. The mean antibody levels were significantly higher for patients with genotype 1 (n = 33) compared with those with genotype 2 (n = 5) (33.2 vs 29.1; P = .007). No correlation was found between antibody levels and severity of hepatic injury with regard to hepatitis activity index or fibrosis score. Six patients with no response to anti-HCV treatment had no change in their mean antibody levels (33.7 vs 34.5). Ten patients who responded to anti-HCV therapy had lower mean levels after therapy, but the changes were not significant (34.2 vs 30.4).

Conclusions: Antibody levels in this study did not correlate with viral load or hepatic injury. However, genotype-2 patients had significantly lower levels compared with genotype-1 patients, and patients who responded to anti-HCV therapy demonstrated decreased antibody levels.

Key words: Hepatitis C recurrence, Liver transplant, Antibody levels
either positive or negative. Because hepatic injury is
due to the host immune response, we felt that quanti-
tative evaluation of the HCV antibody might predict
the severity of hepatic injury and the response to anti-
HCV therapy, and that it may be clinically relevant.
Additionally, it may be particularly important in liver
transplant patients who are immunosuppressed and
may provide a permissive environment for the virus
to multiply in the face of an altered immune response.
We hypothesized that quantitative evaluation of the
HCV antibody may have more clinical significance in
these patients. We therefore sought to quantitatively
estimate the amount of HCV antibody and correlate
this with HCV viral load, genotype, hepatic injury,
response to anti-HCV therapy, and presence of cryo-
globulin in liver transplant patients infected with
HCV.

Materials and Methods

Leftover blood samples originally collected to quanti-
fy hepatitis C viral load were used for quantitative
estimation of HCV antibody levels by using a
VITROS automated hepatitis assay (Ortho-Clinical
Diagnostics, Raritan, New Jersey, USA) [18]. Anti-
body levels were estimated using the signal-to-cutoff
(S/C) ratio. All LTx patients with HCV infection who
attended the LTx clinic at the University of Rochester
Medical Center, Rochester, New York, were given an
opportunity to participate in this study (which had
been approved by our institutional review board); the
study protocol conformed to the ethical guidelines
of the 1975 Helsinki Declaration. Leftover blood
samples from consenting patients that had been
collected for HCV RNA viral load estimations, as
part of their routine follow-up, were used to quanti-
tatively estimate HCV antibody levels.

In all, 141 samples from 39 patients with chronic
HCV infection who underwent LTx between
December 1996 and February 2004 (10 living-donor
liver transplants, 29 deceased-donor liver transplants)
were available for analysis. Five samples from 4
patients were prior to LTx, and the remaining samples
were after LTx. Blood samples that were used for the
study had been collected between October 2001 and
December 2004. Mean age at the time of LTx was
51.76 ± 7.0 years. There were 9 women (23.1%) and 30
men (76.9%). Six patients in this study had genotype
1 with an undetermined subtype, 14 had subtype 1a,
13 had subtype 1b, 4 had subtype 2b, and 1 had geno-
type 2 with an undetermined subtype. In 1 patient,
the genotype was not performed before LTx, and
viral loads were undetectable after posttransplant
treatment.

Thirty-two of 39 patients were given combination
therapy with pegylated interferon 2b or 2a and
ribavirin for the treatment of HCV infection. Those
who did not clear the virus from their plasma (< 50
IU/mL), as detected by polymerase chain reaction
(PCR), after 1 year of treatment were labeled nonre-
sponders. Those who did clear the virus after starting
treatment were labeled responders. Patients who could
not complete the treatment, but achieved a 2 log
reduction in viral load after starting treatment, also
were considered responders for the purpose of
analysis. HCV antibody levels were correlated with
quantitative HCV RNA, HCV genotype and
subtypes, hepatitis activity index (HAI), fibrosis
score, and response to anti-HCV therapy.

Statistical analyses

The results are expressed as means ± standard devia-
tion. Differences in mean antibody levels were
analyzed using the Student t test and one-way analysis
of variance. Differences in mean antibody levels
before and after treatment were compared using the
paired t test. Statistical analyses were performed with
SPSS software (Statistical Package for the Social
Sciences, version 13.0, SSPS Inc, Chicago, Ill, USA).
Values for P less than .05 were considered significant.

Results

The overall antibody levels had a relatively narrow
range from 24.8 to 41.2 except for 4 values, which
were 7.61, 13.3, 13.7, and 17.8. The overall mean was
32.9 ± 5.1 (Figure 1).

Of 141 samples that were for analyzed for quanti-
tative HCV RNA, the upper endpoint of detection
was 0.5 million IU/mL for 21 samples and were
reported as greater than 0.5 million IU/mL. The actu-
al viral load in these samples could not be accurately
determined. In the remaining 120 samples, there was
no apparent correlation between HCV antibody level
and HCV viral load (Figure 1).

In 12 patients (23 samples) with undetectable viral
load (< 50 IU/mL), the mean antibody level was 31.4
± 8.0. The mean antibody level for patients with a
detectable viral load (> 50 IU/mL) was 33.2 ± 4.3; this
difference was not significant (P = .292). Also, the
mean antibody levels for groups based on HCV RNA
levels of ≤ 1 million, > 1 million but ≤ 5 million, > 5
HCV genotype and HCV antibody levels

Genotype information was available for 38 of 39 patients. One patient cleared the virus before entering the study, and genotype was not determined prior to anti-HCV treatment. Of 38 patients, 33 (86.8%) had genotype 1, of whom 6 (15.8%) had an undetermined subtype, 14 (36.8%) had subtype 1a, and 13 (34.2%) had subtype 1b. Their antibody levels were 34.8 ± 3.37 (genotype 1: undetermined subtype), 34.6 ± 4.3 (genotype 1a), and 32.4 ± 6.0 (genotype 1b), respectively. The overall mean HCV antibody level for genotype-1 patients (including all subtypes) was 33.2 ± 4.3 (Table 2). The remaining 5 patients had genotype 2, of which 1 patient had an undetermined subtype, and 4 patients (10.2%) had subtype 2b. The mean antibody levels for these patients were 31.6 ± 1.6 (genotype 2), 28.2 ± 5.3 (genotype 2b), and 29.1 ± 4.7 (overall genotype 2) (Table 2). Overall mean HCV antibody levels were significantly lower for HCV genotype 2 than they were for genotype 1 (P = .007).

Hepatic injury

Thirty-nine patients underwent 47 biopsies during the study. The mean antibody titres were compared in relation to hepatitis activity index (HAI: ≤ 3, > 3, > 6), fibrosis score (0, ≥ 1), and total hepatic injury (Table 1). Mean antibody levels for HAI of ≤ 3 (n = 18), > 3 and ≤ 6 (n = 25), and > 6 (n = 4) were almost identical [34.3 ± 4.0, 34.7 ± 4.2, and 34.7 ± 2.5, respectively (P = .967)]. Similarly, patients with fibrosis scores of 0 (n = 30), or ≥ 1 (n = 17) had almost identical mean antibody levels [35.1 ± 4.4, respectively (P = .513)]. Mean HCV antibody levels for total hepatic injury (combined HAI and fibrosis scores) ≤ 3 (n = 14), > 3 and ≤ 6 (n = 20), > 6 and ≤ 10 (n = 11), or > 10 (n = 2), were also very similar [33.3 ± 3.8, 35.0 ± 4.1, 35.1 ± 4.4, and 34.1 ± 3.2, respectively (P = .790)]. Hepatic activity index, fibrosis score and total hepatic injury against anti-HCV level are shown in Figure 2.

Antibody levels before and after LTx

In 4 patients, antibody levels were available both before and after LTx (Figure 3A). The mean antibody levels before and after transplant in these patients

Table 1. HCV RNA and antibody levels*

<table>
<thead>
<tr>
<th>HCV RNA</th>
<th>Patients</th>
<th>Observations</th>
<th>Mean antibody levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undetectable (&lt; 50 IU/mL)</td>
<td>12</td>
<td>23</td>
<td>31.4 ± 8.0</td>
</tr>
<tr>
<td>Detectable (&gt; 50 IU/mL)</td>
<td>38</td>
<td>118</td>
<td>33.2 ± 4.3</td>
</tr>
<tr>
<td>≤ 1 million</td>
<td>20</td>
<td>38</td>
<td>34.2 ± 4.4</td>
</tr>
<tr>
<td>&gt; 1 million to ≤ 5 million</td>
<td>18</td>
<td>31</td>
<td>32.0 ± 5.0</td>
</tr>
<tr>
<td>&gt; 5 million</td>
<td>15</td>
<td>28</td>
<td>33.2 ± 3.6</td>
</tr>
<tr>
<td>&gt; 0.5 million†</td>
<td>19</td>
<td>21</td>
<td>33.1 ± 3.5</td>
</tr>
</tbody>
</table>

Table 2. Mean antibody levels grouped by genotype

<table>
<thead>
<tr>
<th>Genotype</th>
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<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples (patients)</td>
<td>125 (33)</td>
<td>12 (5)</td>
</tr>
<tr>
<td>Antibody levels</td>
<td>33.2 ± 5.08</td>
<td>29.1 ± 4.22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subtype</th>
<th>U</th>
<th>1a</th>
<th>1b</th>
<th>U</th>
<th>2b</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples (patients)</td>
<td>25 (6)</td>
<td>48 (14)</td>
<td>52 (13)</td>
<td>3 (1)</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Antibody levels</td>
<td>34.9 ± 3.3</td>
<td>34.2 ± 4.3</td>
<td>31.6 ± 4.0</td>
<td>31.6 ± 1.6</td>
<td>28.2 ± 4.5</td>
</tr>
</tbody>
</table>
were 33.9 ± 1.1 and 34.8 ± 1.1 respectively. The difference was not statistically significant ($P = .323$).

**Antibody levels before and after interferon and ribavirin treatment**

Out of 32 patients who received pegylated interferon and ribavirin combination therapy, 18 completed the therapy for 1 year; the other 14 did not complete treatment because of adverse effects. Thirteen of the 32 patients had a response of either clearing the virus or a 2 log reduction in viral load. Of 32 treated patients, antibody levels were available both before and after interferon and ribavirin therapy in 16. Ten of these patients responded to treatment and 6 did not. Of the 10 responders, 5 had mild to moderate decreases in antibody levels, and the remaining 5 had either the same, or a mild rise in, level. Overall mean antibody level in these 10 responders was 34.2 ± 4.4 before anti-HCV therapy, which decreased to 30.4 ± 11.0 (Figure 3B, Table 3, $P = .207$). In 6 patients who did not respond to anti-HCV treatment, the mean antibody level was 33.7 ± 2.6 and did not change much after completion of therapy 34.5 ± 3.8 ($P = .319$) (Figure 3C).

**Antibody levels and cryoglobulinemia**

Of 39 patients, cryoglobulin status was examined in 34. Of these, 31 had no detectable cryoglobulin, while 3 had trace to moderate amounts of cryoglobulin. Patients with positive cryoglobulin had a mean antibody level of 36.8 ± 3.1 while those with negative cryoglobulin had a mean antibody level of 32.4 ± 4.4.

**Discussion**

Until our ability to detect the HCV antibody, the disease was often described as non-A non-B hepatitis. In early 1990s, the HCV antibody was first detected using the ELISA-1 assay (Abbott Laboratories. Abbott Park, Illinois, USA). Later, the ELISA-2 assay was developed, which detected more epitopes of viral
antigen and subsequently, immunoblot assays RIBA-1 and then RIBA-2 techniques were introduced to improve the sensitivity and specificity [19-24]. It became an important test to screen potential blood donors and organ donors to reduce transmission of the HCV virus. It was a major landmark in prevention of disease transmission while providing life-saving treatments. At the same time, development of PCR techniques enabled detection of HCV with almost 100% accuracy. Further developments enabled us to quantify the number of virus copies in peripheral blood. This development rendered antibody testing useful only for screening purposes, and patients testing positive for the antibody underwent PCR for confirmation and quantification of the virus. This development halted further improvements in methods of antibody testing. This interest was recently renewed owing to the possibility of using the HCV antibody for passive immunity against HCV infection, based on the success of hyperimmune hepatitis B globulin (HB Ig). Initial results with HCV immune globulin in chimpanzees were encouraging [25, 26]. However, a randomized trial with human hepatitis-C–immune globulin has failed to provide any benefits in humans [27].

Although the HCV antibody does not provide any protection, it is still a host immune response to infection. It is well known that hepatic damage from HCV varies between subjects. The degree of damage is believed to be due to host response rather than the any direct effect of the virus [15]. The present study was conceptualized to determine if HCV antibody levels correlate with the response to anti-HCV treatment, severity of disease, or hepatic injury. We already know that the immune response in chronic HCV infection is a T-cell–mediated response and therefore, an antibody-mediated response has only limited implications. The primary intent of this study was to examine HCV-infected LTx patients and determine the effect(s) of known predictors of severity and progression of disease (HCV RNA viral load, genotype, and HAI) on fibrosis score on liver biopsy, response to anti-HCV therapy, and effects of immunosuppressive drugs on HCV antibody levels.

Unfortunately, unlike quantitative HCV RNA, the HCV antibody levels exist in a very narrow range in most subjects, and the differences between various groups of known predictors of severity and progression of disease (HCV RNA viral load, genotype, and HAI) studied were not very prominent. Nonetheless, several interesting observations may be taken from this study that may have potential clinical implications and could be further explored. Genotype 2 HCV (mild type) and its subtypes have lower antibody levels than do genotype 1 HCV (aggressive type) and its subtypes. Patients with an undetectable HCV viral load have lower antibody levels compared with those with a measurable viral load, and some of the patients who responded to treatment had a decrease in their antibody levels after anti-HCV therapy. At the same time in this study, antibody levels did not correlate with a higher HCV viral load, hepatic injury, or immunosuppression.

It may be hypothesized from this study that patients with genotype 2 have a more robust T-cell response as compared with patients with genotype 1; therefore, these patients may have a less pronounced B-cell response, and therefore, lower antibody levels. Unfortunately, in this study, we did not have any subjects with genotype 3 for comparison. Further, sequential quantitative antibody level estimations in large populations with longer follow-ups may shed light on these subtle differences. Perhaps a study to access the T-cell response of genotype-2 patients after transplant would be warranted. Nonetheless, it was disappointing not to find a correlation between quantitative viral load and hepatic injury to HCV antibody levels.

Conclusions

HCV antibody levels were distributed within a narrow range in most of our observations. There were no significant differences in antibody levels with regard to viral load, severity of hepatic injury, immunosuppression, or anti-HCV therapy. However, genotype-2 patients had significantly lower antibody levels than did genotype-1 patients. Patients with undetectable viral loads had lower antibody levels compared with those patients with a detectable viral load. Also, some of the patients who responded to anti-HCV treatment had lower antibody levels after therapy. Prospective sequential and multiple measurements of antibody levels in large populations are necessary to establish the utility of the test and its relevance in clinical practice.

References


