Understanding the Presence of False-Positive Antibodies in Acute Hepatitis

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Although false-positive antibodies (FPAs) have been well described in chronic hepatitis C virus (HCV), this has not been evaluated in acute viral hepatitis. Patients with acute viral hepatitis underwent antibody testing for other causes of liver disease and sexually transmitted diseases. Those with antibody positivity underwent confirmatory testing and monitoring. Patients with FPAs were compared with patients with acute hepatitis C infection without FPAs. In total 7 of 24 patients (29%) had FPAs. FPAs during acute viral hepatitis are associated with higher IgM levels and higher ESR in acute HCV. This has both mechanistic and clinical implications and should be evaluated further.

Keywords. acute hepatitis; liver; hepatitis C virus; antibodies; false-positive antibodies.

The vast majority of cases of acute viral hepatitis are due to hepatitis A–E, but other identified etiologies include Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus, varicella zoster virus, and adenovirus [1]. The presentation of acute viral hepatitis is often nonspecific, ranging from the complete absence of symptoms to fulminant hepatic failure [1,2]. In symptomatic patients, the most commonly described symptoms include jaundice, fatigue, abdominal pain, nausea, anorexia, and fevers. Thus, given the poor specificity of presenting symptoms in acute viral hepatitis, identification of the causative factor requires either serologic immunoglobulin (IgM) subclass antibody testing or viral quantification by polymerase chain reaction (PCR) testing. Given that clinical management is purely dependent on the causative factor, accurate diagnosis is paramount, as therapy may prevent chronicity or even death.

The immune response following an acute viral infection is often complex, employing a combination of the innate and humoral immune system [3]. During this process, polyclonal B-cell activation can occur as the host attempts to develop organism-specific antibodies, which is essential for early host defense [4]. This polyclonal B-cell activation typically results from foreign proteins and/or other components of the cell membranes, cytosol, or excreted products from the infecting microorganism and is not specific to any specific viruses, parasites, or bacteria [4]. It has been shown that there can be cross-reactivity between these immune reagents with host “self” antigens, in addition to immune reagents from other infectious organisms, in what has been termed “molecular mimicry” [5]. The presence of organ and non-organ-specific antibodies (NOSAs), including anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (SMAs), rheumatoid factor (RF), antimitochondrial antibodies (AMAs), and anti-liver kidney microsomal (LKM) antibodies, has been well described in many infections, including chronic HCV, hepatitis B virus (HBV), and human immunodeficiency virus (HIV) [6,7]. Indeed, studies have shown the presence of NOSAs in up to 70% of patients with chronic HCV [8], and it is felt that the process of molecular mimicry may be responsible for the multiple extrahepatic complications that are often autoimmune mediated, such as mixed cryoglobulinemia, lichen planus, and non-Hodgkin B-cell lymphoma [3].

In addition to these auto-antibodies, false-positive IgM responses toward other viruses have also been associated with many infectious agents [9-11] and even vaccinations [12]. False-positive IgM for EBV and CMV have been reported to occur in approximately 3% of patients with acute HIV, and up to 30% of patients with acute hepatitis A infection [13]. Of interest, there is a single case report of a false-positive HIV test in a patient with acute Q fever-associated hepatitis [14]. There is also evidence to suggest up to 4.5 times higher prevalence of biologic false-positive tests for syphilis in patients with chronic HCV [9]. These findings are significant, as many of these infections share many of the same risk factors and have similar clinical presentations, and accurate diagnosis is crucial to proper treatment.

The presence of these auto-antibodies and FPAs is felt to be rare in acute hepatitis [15] and thus has not been well described and their significance is unclear. We present a series of cases of
acute hepatitis that were associated with the presence of NOSAs and FPAs.

METHODS

A total of 24 patients who presented to the Liver Diseases Branch of the National Institutes of Health (NIH) for evaluation of acute hepatitis were included in this study. Twenty-two patients with a diagnosis of acute hepatitis C were previously described in a study by Loomba et al [2]. In addition, 1 patient was found to have acute hepatitis B, and a second patient was found to have acute hepatitis due to CMV.

All patients were evaluated for other causes of chronic liver disease, including other viral causes, autoimmune disease, medications, and metabolic causes when appropriate. Laboratory analysis included checking antibodies to hepatitis A, B, C, D, and E, HIV, human T-cell lymphotrophic virus (HTLV), CMV, EBV, varicella zoster virus (VZV), herpes simplex virus (HSV), rapid plasmin reagent (RPR), and fluorescent treponema antibody (FTA) abs, in addition to ANA, SMA, AMA, LKM, RF, C-antineutrophil cytoplasmic antibody (C-ANCA), P-antineutrophil cytoplasmic antibody (P-ANCA), and immunoglobulins. Patients in the original study were begun on treatment for acute HCV with interferon and ribavirin-based regimens according to the standard of care at that time if they did not show signs of clearing the infection after a few weeks.

Patients with antibodies towards other infections had serial or confirmatory testing done to ensure that they were false positives (HIV had Western blot and PCR; HTLV had Western blot; RPR had FT-ABS). Patients who had NOSAs and FPAs at time of initial presentation were then evaluated to see if they had loss of these antibodies. These patients were included in the “abnormal antibody” group. The remaining patients were included as controls. As interferon itself has been associated with inducing or unmasking underlying autoimmune diseases in patients, only positive antibodies prior to treatment with interferon were used.

The 2 groups were then compared in regards to liver function tests, viral loads, immunoglobulin levels, and erythrocyte sedimentation rate (ESR). Labs were added on to stored samples when necessary. Viral loads were only compared between patients with acute HCV.

Charts and statistical analysis was performed using Prism Graphpad software (version 5.0f), and P values were calculated using an unpaired t-test. A separate analysis was also performed using only the patients with acute HCV.

RESULTS

Table 1 shows the patient demographics for both groups. Of the 24 patients evaluated for acute hepatitis, a total of 7 (29%) had NOSAs and/or false-positive antibodies at the time of diagnosis: 5 (71.4%) with acute hepatitis C, 1 with acute hepatitis B, and 1 with acute CMV hepatitis. All patients in the control group had acute hepatitis C. In patients with acute HCV, detected FPAs included HIV, HTLV, anti-smooth muscle, rheumatoid factor, and RPR. In the patient with acute HBV, rheumatoid factor was the NOSA detected and in the patient with acute CMV, hepatitis E IgM was the FPA detected.

In sum, 57.1% of the patients with FPAs were male, compared to 41.2% in the control group. Median age (43 vs 39 years, P = .348) and presence of symptoms at the time of diagnosis (71.4% vs 70.5%, P = .483) was similar between the 2 groups. No patients with FPAs were infected via occupational exposure, compared to 53% of the control group (P = .009). In sum, 100% of the patients with FPAs eventually resolved their infection (with or without treatment) compared to 76.5% of the control group (P = .084). Treatment rates for hepatitis C were similar between both groups (80% vs 76.4%).

Supplementary Table 1 lists the patients who had NOSAs and FPAs at the time of diagnosis. The antibodies were lost between 2 weeks after diagnosis and up to 1 year after sustained virologic response.

At the time of diagnosis, patients with FPAs had significantly higher median IgM levels compared to those without FPAs (292 vs 131 mg/dL, P = .002; Supplementary Table 2). However, at the time of FPA resolution, IgM levels were no longer significantly different between groups (Figure 1). Patients also had higher ESR levels at the time of diagnosis compared to those without FPAs (31 vs 19.5 mm/hour, P = .003; Supplementary Figure 1). Serum cryoglobulins were assessed in all patients at the first visit, and a single positive result was found in each group.

Table 1. Characteristics of Study Cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Abnormal Antibody n (%)</th>
<th>Control n (%)</th>
<th>Total n (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of patients</td>
<td>7</td>
<td>17</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Acute hepatitis C</td>
<td>5 (71.4)</td>
<td>17 (100)</td>
<td>22</td>
<td>.076</td>
</tr>
<tr>
<td>Acute CMV</td>
<td>1 (14.3)</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Acute HBV</td>
<td>1 (14.3)</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4 (57.1)</td>
<td>7 (41.2)</td>
<td>11 (45.8)</td>
<td>.242</td>
</tr>
<tr>
<td>Median age at diagnosis</td>
<td>43 ± 16</td>
<td>39 ± 16</td>
<td>39 ± 17</td>
<td>.348</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>5 (71.4)</td>
<td>12 (70.5)</td>
<td>17 (75)</td>
<td>.483</td>
</tr>
<tr>
<td>Cleared infection</td>
<td>7 (100)</td>
<td>13 (76.5)</td>
<td>21 (85.5)</td>
<td>.084</td>
</tr>
<tr>
<td>Mode of transmission</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2 (28.6)</td>
<td>3 (17.6)</td>
<td>5 (20.8)</td>
<td>.278</td>
</tr>
<tr>
<td>IVDU</td>
<td>1 (14.3)</td>
<td>1 (5.9)</td>
<td>2 (8.3)</td>
<td>.507</td>
</tr>
<tr>
<td>Occupational</td>
<td>0</td>
<td>9 (52.9)</td>
<td>9 (37.5)</td>
<td>.009</td>
</tr>
<tr>
<td>Other</td>
<td>2 (28.6)</td>
<td>3 (17.6)</td>
<td>5 (20.8)</td>
<td>.278</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (28.6)</td>
<td>1 (5.9)</td>
<td>3 (12.5)</td>
<td>.194</td>
</tr>
</tbody>
</table>

Abbreviations: CMV, cytomegalovirus; HBV, hepatitis B virus; IVDU, intravenous drug use.

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Median viral loads at the time of diagnosis and peak viral loads were compared for the patients with acute hepatitis C only. Although peak viral loads were higher in the control group compared to the FPA group, results were not significant (18,148 vs 102,000, \( P = .135 \)). Differences between mean and peak false positive antibody (ALT) and aspartate aminotransferase (AST) were also not significant between the groups.

**DISCUSSION**

Although the association between NOSAs and chronic hepatitis is well documented, it was previously felt not to be significant in acute hepatitis. Our findings suggested that acute hepatitis is also associated with the production of NOSAs, in addition to FPAs to other viruses, which clear after resolution of the acute infection. This includes antibodies to diseases that may complicate the diagnosis and treatment, such as in the case of the false-positive HIV antibodies.

These false-positive antibodies are felt to be due to a strong immune response to the infecting agent and the subsequent polyclonal B-cell activation as the host attempts to clear it. It is therefore not unexpected that we should find higher values of IgM and ESR in the patients who were found to have NOSAs and false-positive antibodies. However, the significance of this difference is unclear.

Another interesting finding was that none of our patients in our study group were infected through occupational exposure but rather through higher risk methods (IV drug use, sexual transmission), whereas over half of the control group were infected through occupational exposure (\( P = .009 \)). It has previously been noted that there is a higher biological false-positive rate for syphilis in intravenous drug users [9]. Ironically, it is these patients who are also at higher risk for coinfections with these other infections, and thus awareness that these positive tests may be false is important.

Our study was limited by the fact that it is a case series with a small sample size, which potentially affected the significance of the laboratory findings. Additionally, our control group consisted solely of patients with acute HCV infections, whereas we had 1 patient with acute hepatitis B and another with acute CMV hepatitis in the study group. The significance of the differences in immune responses for these viruses, in addition to any differences in the effects of molecular mimicry cannot be determined by our study.

**CONCLUSION**

Although the presence of NOSAs has been well established in chronic HCV, the significance of these, in addition to other false-positive antibodies, has not been previously well studied. Serologic detection of FPAs during acute viral hepatitis is likely associated with higher viral inoculums, as well as higher IgM levels and nonspecific markers of inflammation. This has both mechanistic and clinical implications and should be evaluated further.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**References**