Review

Clinical applications of antibody avidity and immunoglobulin M testing in acute HCV infection

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Acute hepatitis C is often asymptomatic, frequently remains undiagnosed and frequently evolves to chronic hepatitis. Early, short-term interferon treatment is efficacious in acute hepatitis C, and so underscores the importance of an early diagnosis and the need to distinguish acute infection from acute exacerbation of chronic HCV infection. The gold standard for the diagnosis of acute hepatitis C is demonstration of conversion to anti-HCV positivity, HCV RNA positivity or both, events that frequently occur before the patient comes to medical attention. Several laboratory approaches to assist with early diagnosis of acute hepatitis C have been developed. Our studies, reviewed here, show that testing for antibody avidity and anti-HCV immunoglobulin M allow diagnosis in up to 90% of cases of acute hepatitis C.

Introduction

Acute hepatitis caused by HCV is characterized in its symptomatic form by nausea, malaise, abdominal pain and jaundice [1]. In most cases, HCV transmission follows percutaneous exposure to human blood, but more recently increasing evidence has highlighted unsafe sexual intercourse [2–4], particularly in HIV-positive males who have sex with males [5–8]. Worthy of note is the changing impact of various risk factors for HCV transmission in the last 20 years in several countries, consequent to variations in social and economic conditions and to the widespread use of invasive medical procedures, particularly in developing countries [2,3,9,10].

The incidence of acute hepatitis C (AHC) has recently declined in the USA, Western Europe and Australia [9,11], but it still remains a serious, unsolved health problem in most areas of the world. AHC is frequently asymptomatic, with one-third of patients spontaneously clearing the infection and the remaining two-thirds needing treatment to prevent the progression to chronicity. The outcome of AHC can vary from viral clearance with the consequent remission of acute illness to persistence of the infection associated with progression to chronic disease. Both viral factors (viral quasispecies and HCV genotype) [12–14] and host factors (sex, initial immune response, and interleukin-28B genotype) have been reported to be involved in disease progression [15–22]. HCV-related chronic hepatitis is characterized by the persistence of both anti-HCV and HCV RNA peripheral blood, and adopts an indolent course, but in about one-third of cases is associated with slow progression to liver cirrhosis and hepatocellular carcinoma [1].

Need for early and correct diagnosis of acute hepatitis C

Because AHC is frequently asymptomatic, it often remains undiagnosed [1]. This feature stresses the need for prompt treatment to retard the progression to chronicity [23]. Jaeckel et al. [24] reported the eradication of HCV in 98% of patients with AHC who received an early, 6-month standard course of interferon treatment. The favourable effect of early treatment was confirmed by several authors [24–26]. In particular, Nomura et al. [25] found that patients who started the standard interferon treatment within the first 8 weeks from the onset of symptoms had a significantly higher sustained virological response (SVR) rate than those who started treatment one year after onset. A randomized clinical trial demonstrated that a 3-month pegylated interferon-α2b treatment started at week 8 or 12 resulted in higher SVR rates than therapy started at week 20 (95% and
92% versus 76%, respectively) [25]. Higher SVR rates were found in patients infected with HCV genotypes 2, 3 and 4 than those infected with genotype 1. It has also been demonstrated that in patients with AHC, the addition of ribavirin to pegylated interferon did not improve the SVR rate [26].

The favourable effect of early interferon treatment in AHC not only stresses the importance of early diagnosis but also the need to distinguish it from acute reactivation of chronic HCV infection. Acute reactivation is a frequent event in chronic HCV infection and has a clinical presentation similar to that of acute hepatitis [27–31], but requires the same treatment applied to patients with chronic hepatitis C. In addition, subjects with chronic hepatitis C with persisting risk factors for acquisition of HCV infection, for example, injecting drug users, may undergo a new episode of AHC due to a different HCV strain whose clinical picture may again be indistinguishable from an acute reactivation of chronic HCV infection [32–37].

**Role of anti-HCV immunoglobulin G avidity and anti-HCV immunoglobulin M**

The gold standard for the diagnosis of AHC is still the conversion to anti-HCV positivity, HCV RNA, or both. Seroconversion is, however, infrequently detected in everyday clinical practice because most patients with AHC do not know their previous anti-HCV/HCV RNA status [38]. Therefore, several attempts have been made to develop other approaches to the diagnosis of AHC.

Araujo et al. [39] developed a flow-cytometric microsphere immunoassay to measure anti-HCV immunoglobulin (Ig)G reactivity specific to recombinant core NS3, NS4 and NS5 HCV proteins and applied it to serum samples from anti-HCV seroconverters and to subjects with chronic infection. These authors found a significant difference in the reactivity to each antigen tested between the two groups of patients; multivariate logistic regression model correctly classified the samples in the two groups, with a cross-validation accuracy of 91% for the acute group and 97% for the chronic group.

The avidity of specific IgG is low in the early phase of several viral infections, but it increases with time [40–44]. For this reason, our group and other workers have developed methods to detect anti-HCV IgG avidity to distinguish acute from chronic HCV infection [45,46]. Gaudy-Graffin et al. [47] used urea to break the low-strength binding between the IgG and their corresponding antigens. They tested sera from patients with acute, chronic or resolved HCV infections and were able to distinguish patients with acute from those with chronic hepatitis C with high sensitivity (98%) and specificity (100%).

In our previous studies [48,49] we evaluated serologic changes in avidity of anti-HCV IgG and anti-HCV IgM serially in AHC. We enrolled symptomatic patients with AHC diagnosed on the basis of conversion to anti-HCV positivity, and symptomatic patients with acute exacerbation of chronic hepatitis C diagnosed on the basis of the persistence of anti-HCV in serum for at least one year before reactivation. In the first study, performed to explore the usefulness of avidity of IgG to diagnose AHC [49], guanidine was used to break the binding between IgG and its corresponding antigens, modifying a commercial assay. For each serum sample the HCV avidity index (AI) was calculated by dividing the optical density (OD) of the guanidine-diluted sample by the OD obtained with the same serum diluted with PBS. Of the 40 patients with AHC investigated, the AI was lower than 0.7 in all 15 patients first observed within eight days from the onset of symptoms, in 11 (64.7%) of the 17 patients first tested at days 9–11, and in 1 (12.5%) of the 8 patients first tested later. Of 37 patients with reactivation of chronic hepatitis C, an HCV AI lower than 0.7 was found in 3 (50%) of 6 drug users first tested 5–8 days after the onset of symptoms, in none of 10 drug users first tested later and never found in 21 patients with no history of drug addiction; for the 3 drug users with an AI lower than 0.7 a possible superinfection by a second HCV viral strain was not ruled out. For a comprehensive evaluation, 40 patients with chronic hepatitis C without exacerbation, pair-matched by age and sex with the 40 patients with AHC, were also tested. An AI<0.7 was never found. In patients with AHC, there was a tendency for AI to increase rapidly from this value in the subsequent weeks.

It is a common knowledge that a single determination of IgM to HCV core protein does not discriminate between acute and chronic hepatitis C [50–57] since anti-HCV IgM may be present also in patients with chronic hepatitis C. Consequently, we made an attempt to diagnose acute hepatitis C by serial determination of anti-HCV IgM [48]. Briefly, IgM to the HCV core protein was determined by a commercial enzyme immunoassay where serum samples showing a sample to cutoff ratio ≥1 are considered positive. By this method IgM to HCV core protein can be quantified as an index value (IV). IgM titres were detected at two or three checking points during the initial 2–3 weeks of the acute phase of illness and a marked difference was found between 35 patients with AHC and 31 with reactivation of chronic hepatitis C, whereas much variation was found in patients with AHC: an initial marked increase followed by a rapid subsequent decrease in IV was observed in 8 patients; a clear increase starting from a low or intermediate
IV was observed in 11 patients, and a rapid decrease starting from a high IV was observed in the remaining 16. Consequently, the diagnosis of AHC was identified on the basis of a significant increase followed by a subsequent rapid decrease in the IV in 23% of cases, of a significant increase in 31% and of a decrease in 46% (Table 1). The variability of the IgM response in patients with AHC probably reflects a variety of interactions between HCV and the infected host.

The results of these two studies were encouraging, but the need for serial avidity and IgM determinations was a limitation to application in routine clinical practice. Consequently, we tried to improve the diagnostic power of these methods by determining both the IgM titre and IgG avidity on the same serum sample. Forty-five patients with AHC and 36 patients with reactivation of chronic hepatitis C with no history of drug addiction were investigated [58]. Specific cutoff values were identified for four selected time points. For the HCV IgM assay, the highest value observed in chronic hepatitis C patients with reactivation plus 5%; for the HCV avidity assay, the lowest value observed for the same patients minus 5%. In sera obtained within 15 days from the onset of symptoms, IgG avidity was more sensitive than the IgM titre to diagnose AHC (Table 2), suggesting that the IgG avidity should be performed as the first test in all patients and the IgM titre as the second test only in samples that gave equivocal results with avidity testing. Using this approach, we could diagnose AHC in 93% of samples collected by the 10th day from the onset of symptoms and in 86% collected between the 11th and 15th days. By contrast, for sera obtained after the 15th day, the more sensitive test was the IgM titre, suggesting that the IgM titre should be performed as the first test in all samples and IgG avidity as the second test in samples giving equivocal IgM results. By this procedure, we confirmed the diagnosis of AHC.

**Table 1.** IgM to HCV titres in the 35 patients with acute hepatitis C, by the different behaviour of IgM to HCV titres, and in the 31 patients with reactivation of chronic hepatitis C

<table>
<thead>
<tr>
<th>Patients</th>
<th>5–8 days&lt;sup&gt;a&lt;/sup&gt;</th>
<th>9–11 days&lt;sup&gt;a&lt;/sup&gt;</th>
<th>12–15 days&lt;sup&gt;a&lt;/sup&gt;</th>
<th>16–18 days&lt;sup&gt;a&lt;/sup&gt;</th>
<th>19–22 days&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 Patients with AHC and increase and a subsequent decrease of IgM HCV IV Number of samples tested</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>11 Patients with AHC and increase of IgM HCV IV Number of samples tested</td>
<td>125 (91–166.5)</td>
<td>541 (294–685)</td>
<td>775 (637.5–1015)</td>
<td>1,000 (1,000–1,000)</td>
<td>–</td>
</tr>
<tr>
<td>16 Patients with AHC and decrease of IgM HCV IV Number of samples tested</td>
<td>2,85 (794–1,812.5)</td>
<td>666 (440–757)</td>
<td>396 (290–524.5)</td>
<td>203 (62.25–560)</td>
<td>80 (45–132)</td>
</tr>
<tr>
<td>31 Patients with reactivation of chronic hepatitis C Number of samples tested</td>
<td>73 (41–320)</td>
<td>79 (45–185)</td>
<td>83 (47–183)</td>
<td>88 (46–119)</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are immunoglobulin (Ig)M HCV sample to cutoff ratio median (IQR) unless otherwise indicated. *From onset of symptoms. AHC, acute hepatitis C; IgM HCV IV, IgM HCV index value.

**Table 2.** Improvement in the aetiological diagnosis of acute hepatitis C: a diagnostic protocol based on the anti-HCV-IgM titre and IgG avidity index

<table>
<thead>
<tr>
<th>Days from onset of symptoms</th>
<th>Test</th>
<th>AUC</th>
<th>Cutoff</th>
<th>Sensitivity, number positive/number tested (%)</th>
<th>Diagnostic algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>IgM</td>
<td>0.946</td>
<td>73.5 IV</td>
<td>14/26 (53.8)</td>
<td>IgG-Al</td>
</tr>
<tr>
<td></td>
<td>IgG-Al</td>
<td>0.907</td>
<td>0.62 AI</td>
<td>22/26 (84.6)</td>
<td></td>
</tr>
<tr>
<td>11–15</td>
<td>IgM</td>
<td>0.903</td>
<td>50.4 IV</td>
<td>21/29 (72.4)</td>
<td>IgG-Al</td>
</tr>
<tr>
<td></td>
<td>IgG-Al</td>
<td>0.908</td>
<td>0.77 AI</td>
<td>22/29 (75.9)</td>
<td></td>
</tr>
<tr>
<td>16–20</td>
<td>IgM</td>
<td>0.992</td>
<td>24.8 IV</td>
<td>24/27 (88.9)</td>
<td>IgM</td>
</tr>
<tr>
<td></td>
<td>IgG-Al</td>
<td>0.921</td>
<td>0.80 AI</td>
<td>18/27 (66.7)</td>
<td></td>
</tr>
<tr>
<td>&gt;20</td>
<td>IgM</td>
<td>0.802</td>
<td>18.9 IV</td>
<td>12/16 (75.0)</td>
<td>IgM</td>
</tr>
<tr>
<td></td>
<td>IgG-Al</td>
<td>0.885</td>
<td>0.87 AI</td>
<td>9/16 (56.2)</td>
<td></td>
</tr>
</tbody>
</table>

Data from [58]. *The second test was performed only in patients not diagnosed with the first. AI, avidity index; AUC, area under curve; Ig, immunoglobulin; IV, index value.
in 93% of samples obtained between the 16th and 20th days after the onset of symptoms, and in 88% of those collected after the 20th day (Table 2). Overall, of 45 patients with AHC, 7 were not correctly diagnosed at one of the four selected time points. Of these seven patients, six were correctly diagnosed at the previous or next time points; thus, only one patient with a single serum sample obtained 25 days after the onset of symptoms remained undiagnosed, confirming the diagnostic power of the combination of these two tests on a single serum sample. Further evaluation of these approaches in other clinico-epidemiological settings is needed to confirm these encouraging results.

Anti-HCV avidity and immunoglobulin M testing in perspective

In an anti-HCV-negative person at risk of acquiring HCV infection, it may be possible to conduct periodic screening that includes physical examination and testing for elevated liver aminotransferases and presence of anti-HCV in serum. A person unaware of the previous anti-HCV status but with incidental aminotransferase serum levels >200 IU/ml could be considered to be experiencing acute hepatitis or reactivation of chronic hepatitis. For a person who is known previously to be anti-HCV-negative, seroconversion is a reliable indicator of AHC. For a patient who is an anti-HCV-positive patient, the determination of IgM titres and IgG AI becomes of value in distinguishing AHC from reactivation. Figure 1 depicts how avidity and IgM testing in serum may be incorporated into the algorithm for identifying and treating patients with AHC. It incorporates characterization of polymorphisms in the patient’s interleukin 28-B gene [59–61].

Acknowledgements

This study was supported by a grant from Progetti di Ricerca Scientifica di Rilevante Interesse Nazionale 2008, Ministero dell’Istruzione e della Ricerca Scientifica, Rome, Italy, ’Improvement in diagnosis of acute hepatitis C and identification of immunological factors inducing recovery of chronic HCV infection’.

Disclosure statement

The authors declare no competing interests.

References

Antiviral Therapy 17.7 Pt B


