The diagnosis of recent HCV infection remains challenging due to the absence of serological markers specific to the early phase of infection. Clinical follow-up and seroconversion to anti-HCV immunoglobulin (Ig)G, detection of viral RNA and changes in levels of blood biomarkers associated with liver pathology provide circumstantial evidence of recent HCV infection. Studies based on anti-HCV IgG avidity, antigen-specific antibody profiling, HCV viral load fluctuations and signature changes in the HCV genome show potential to discriminate recent from persistent HCV infection. These markers require further evaluation and would necessitate use of samples from infected people originating from broad clinical and epidemiological contexts.

The diagnosis of acute HCV infection is based on the demonstration of seroconversion to anti-HCV immunoglobulin (Ig)G in an infected person and the serological appearance of HCV RNA, which may or may not persist after the acute phase has passed [1]. These two markers of HCV infection are often detectable in patients at the time they present with clinical or laboratory evidence of hepatitis C. Additionally, liver function tests cannot be used to specifically indicate recent HCV infection. Moreover, increases in serum levels of aminotransferases of hepatic origin such as alanine aminotransferases (ALT) can occur during flare-ups or exacerbation of chronic disease [2–4], which may confuse diagnosis. Thus, factors such as history of recent exposure to HCV and appearance of symptoms need to be additionally considered.

The diagnosis of recent HCV infection is relatively easier in some specific risk groups, such as health-care workers in whom the time of exposure and seroconversion may be documented [5,6]. By contrast, it is challenging to diagnose recent infection in injecting drug users (IDUs). Acute hepatitis C is frequently asymptomatic [7,8] and even when symptoms appear, IDUs might hesitate to seek medical assistance due to cost and social stigma [9].

Detection of recent HCV infection is clinically important because treatment is considerably more efficacious if started during the acute phase. Early identification followed by treatment with interferon-α (INF-α) and ribavirin can lead to eradication of the virus in up to 98% of cases [10,11], compared with 40–80% of cases when treatment starts after chronic infection has been established [12,13].

Antibody-based approaches

Researchers have attempted to develop a test for anti-HCV IgM [2,14–17] or construct algorithms that include IgM titres and IgG avidity [18]; however, IgM antibodies to HCV also are present during the chronic phase of infection. The correlation, albeit weak, between serological detection of anti-HCV IgM and appearance of various clinical and laboratory features of HCV infection has been reported in several studies involving patients with chronic hepatitis C. Anti-HCV IgM positivity was observed in one study to denote active HCV-induced liver disease, and its disappearance during INF-α therapy could be used as a predictor of sustained virological response [19]. In another study, the presence of HCV core IgM antibodies prior to treatment with INF-α prognosticated failure of viral clearance [20]. Some other studies indicated that anti-HCV IgM could be utilized as a marker of HCV replication, reactivation or persistence of chronic infection [3,21–23]. The value of anti-HCV IgM tests for clinical purposes remains questionable because these observations are reported only from studies involving small sample sizes and several different IgM assays were used (commercial and
‘in-house’), whose performance characteristics have not been fully evaluated. Studies in larger and more diverse groups are required to evaluate these assays further.

Other researchers have used variation in HCV antibody titres for the detection of acute HCV infection [4,18,24,25]. In one prospective study that included 18 patients with acute infection and 4 with acute exacerbation during chronic infection, the trend in the signal/cutoff ratio (SCR) was documented between weeks 12 and 24 after ALT increase. It was observed that 86% of the subjects with acute infection showed an increase in SCRs, but they remained stable in the subjects with chronic infection [4]. In another study, Nikolaeva and colleagues [24] reported that in acute infection the titres of anti-core IgG were ≤1/800 and anti-non-structural (NS) proteins were <1/800 also, while in the chronic phase the titres of anti-core IgG were ≥1/800 (1/40,000, the highest) and against the NS proteins were ≥1/5 (1/20,000, the highest). By contrast, in a study that examined samples of 12 IDUs before, during and after infection, Netski and collaborators [25] reported that the levels of antibody responses to NS proteins were higher than those to the structural proteins.

Another approach based on antibody titre is the measurement of adding a chaotropic reagent, such as urea or guanidine, prior to immunoassay for antigen-specific IgG. These agents interfere with stabilizing intermolecular forces between antigen–antibody causing the disruption of non-covalent bonds. This approach has been used for many years for the detection of several microbial infections during the acute stage [26–28] and to discriminate recent from past infections [29,30]. Several authors have reported the use of commercial enzyme immunoassays (EIA) modified to improve diagnosis of recent HCV infection [31–34].

We have developed an anti-HCV IgG avidity assay using a mixture of recombinant HCV proteins (core, NS3 and NS4) as antigens and urea as the chaotropic agent [35]. EIA was performed in duplicate wells and after sample incubation and washing, one of the wells was treated with 0.85% NaCl and the other with 8 M urea. AI was calculated as a percentage using the ratio of the optical density value obtained after urea treatment divided by the value obtained with 0.85% NaCl treatment. We determined that an AI cutoff value <42% indicates acute infection. Among seroconverting blood donors, all samples taken from the early acute stage (obtained with <65 days after seroconversion to anti-HCV IgG) could be discriminated from samples obtained during the chronic stage using that AI cutoff value. The mean AI for samples obtained with >65 days after seroconversion (that is, during the late acute stage) was 63%, reflecting an increase in anti-HCV IgG antibody titres that occurs with progression of the disease; the difference between the AI from samples obtained during the late acute phase and during resolved infection was not statistically significant. The AI approach needs further evaluation in various clinical settings.

More recently, we have developed a multiplexed immunoassay to measure specific anti-HCV IgG reactivities by using a spectrally distinct bead-based technology, with each bead-set carrying one HCV recombinant antigen (core, NS3, NS4 and NS5). Beads coupled to these antigens were mixed, incubated with serum, and the reactivities to these antigens differentiated in an analyser similar to a flow cytometer (Luminex, Austin, TX, USA). HCV-specific antibodies bound to the antigens were detected by fluorescent reagent [36]. Using serum samples from seroconversion panels and chronically infected donors, we observed that for all the antigens tested the SCRs were significantly higher in samples from donors with chronic infection. The differences in SCRs between the acute and chronic sample groups are due to increasing IgG antibody titres occurring in the course of infection. Based on these differences a multivariate logistic regression model was built; cross-validation showed that the model was 91% accurate for the acutely infected group and 97% for the chronically infected group. The Luminex-based assay detected HCV antibody response in the acute samples earlier than the anti-HCV IgG avidity assay and a recombinant immunoassay. Therefore, besides being able to identify recent HCV infection, this high-throughput microsphere-based immunoassay has potential to shorten the ‘window period’ of infection [36]. A further advantage of the multiplexed flow cytometric microsphere immunoassay is its ability to determine IgG reactivity specific to each of the HCV recombinant proteins. Moreover, it only requires testing of a single specimen, can be quickly performed and because a very small sample volume is required, allows for testing of a large number of specimens. Further evaluation of this approach in various clinical settings is required for validation. Table 1 summarizes the immunological assays thus far developed for the serological diagnosis of acute HCV infection.

The existence of HCV proteins encoded by alternate reading frames (ARFP) overlapping the core gene has been reported in several studies [37–39]. Morice and colleagues [40] have shown seroconversion to the ARFP during acute infection. However, these findings are not conclusive because other authors have shown reactivities to ARFP-derived peptides during the chronic phase of infection [37].

**Genome-based approaches**

Recently, McGovern and colleagues [41] have reported that the diagnosis of acute infection in HCV-seropositive patients can be improved by adding information on
### Table 1. Immunological assays for detection of acute HCV infection

<table>
<thead>
<tr>
<th>Type of assay</th>
<th>HCV antigens</th>
<th>Genotype</th>
<th>Protein expression system</th>
<th>Manufacturer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified anti-HCV for IgM detection</td>
<td>Recombinant NS4</td>
<td>NA</td>
<td>NA</td>
<td>Ortho Diagnostics Systems, Berne, Belgium</td>
<td>[17]</td>
</tr>
<tr>
<td>Immunoblotting</td>
<td>Core (AA 1–120)</td>
<td>NA</td>
<td>Escherichia coli</td>
<td>'In-house'</td>
<td>[2]</td>
</tr>
<tr>
<td>Modified EIA for anti-HCV IgG avidity</td>
<td>Core (AA 1–120)</td>
<td>NA</td>
<td>NA</td>
<td>United Biomedical Inc., New York, NY, USA</td>
<td>[34]</td>
</tr>
<tr>
<td>Third-generation EIA (antibody titre for IgG and IgM)</td>
<td>Core, NS3, NS4 (A + B), NS5A (full length)</td>
<td>1A</td>
<td>NA</td>
<td>Vector Best, Novosibirsk, Russia</td>
<td>[24]</td>
</tr>
<tr>
<td>Modified EIA for anti-HCV IgG avidity</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Ortho Clinical Diagnostics, Piscataway, NJ, USA</td>
<td>[31]</td>
</tr>
<tr>
<td>Third-generation anti-HCV EIA (AxSYM, version 3.0)</td>
<td>Core (AA–150), NS3 (AA 1192–1457)</td>
<td>NA</td>
<td>Escherichia coli</td>
<td>Abbott Laboratories, Chicago, IL, USA</td>
<td>[4]</td>
</tr>
<tr>
<td>Anti-HCV IgM titre (HCV IgM EIA 2.0)</td>
<td>NA</td>
<td>NA</td>
<td>Saccharomyces cerevisiae</td>
<td>Chiron, Emeryville, CA, USA</td>
<td>[25]</td>
</tr>
<tr>
<td>'In-house' EIA for antigen-specific IgG responses</td>
<td>Core (AA 2–120), NS4 (AA 1569–1931), NS5 (AA 2054–2995) NS3 (AA 1192–1457)</td>
<td>NA</td>
<td>Yeast as superoxide dismutase fusion proteins Escherichia coli</td>
<td>Vero cells</td>
<td>[26]</td>
</tr>
<tr>
<td>Anti-HCV IgG avidity</td>
<td>Core (AA 1–100), NS3 (AA 1192–1459)</td>
<td>1B</td>
<td>Escherichia coli as a chimera with glutathione S-transferase</td>
<td>RPC Diagnostic Systems, Nizhniy Novgorod, Russia</td>
<td>[35]</td>
</tr>
<tr>
<td>Modified commercial EIA for anti-HCV IgG avidity</td>
<td>NA</td>
<td>NA</td>
<td>Saccharomyces cerevisiae</td>
<td>Chiron, Emeryville, CA, USA</td>
<td>[25]</td>
</tr>
<tr>
<td>'In-house' EIA-based anti-HCV IgG avidity assay</td>
<td>Core (AA 1–100), NS3 (AA 1192–1459)</td>
<td>1B</td>
<td>Yeast as superoxide dismutase fusion proteins Escherichia coli</td>
<td>Vero cells</td>
<td>[26]</td>
</tr>
<tr>
<td>Combination of modified EIAs for anti-HCV IgG avidity and IgM titres</td>
<td>NS3 (AA 1356–1459), Mosaic NS4 (1691–1710, 1712–1733, 1921–1940)</td>
<td>1A, 1B, 1C, 2C</td>
<td>NA</td>
<td>Ortho Clinical Diagnostics, Rochester, NY, USA and Equipar Diagnostics, Saronno, Italy</td>
<td>[18]</td>
</tr>
<tr>
<td>Modified EIAs for anti-HCV avidity</td>
<td>NA</td>
<td>NA</td>
<td>Saccharomyces cerevisiae</td>
<td>Ortho Clinical Diagnostics, Piscataway, NJ, USA</td>
<td>[33]</td>
</tr>
<tr>
<td>'In-house' multiplexed, flow cytometric microsphere immunoassay to measure individual anti-HCV IgG reactivities</td>
<td>Core (AA 1–100), NS3 (AA 1192–1459)</td>
<td>1B</td>
<td>Escherichia coli as a chimera with glutathione S-transferase</td>
<td>RPC Diagnostic Systems, Nizhniy Novgorod, Russia</td>
<td>[36]</td>
</tr>
</tbody>
</table>

AA, amino acid; EIA, enzyme immunoassay; Ig, immunoglobulin; NA, information not available; NEIBM, novel evolved immunoglobin molecule.
viral load fluctuations and low levels of HCV RNA into the diagnostic criteria. The authors analysed samples of a cohort of 37 IDUs diagnosed with acute HCV infection and observed that the viral load fluctuations were >1 log and/or the levels of viraemia were low (<10^5 IU/ml). Although useful in various clinical settings, this approach requires serial monitoring of HCV RNA levels for 10–12 weeks.

Another line of research has been the correlation of viral evolution with different stages of HCV infection or the outcome of disease. In a longitudinal study involving four subjects, whole-genome HCV sequences were obtained during a period of 30 months after onset of symptoms. Regardless of viral load, the mutation rate was highest during the acute phase of infection, decreasing thereafter [42]. Additionally, several studies have shown that the rate of non-synonymous mutations in the HCV hypervariable region 1 (HVR1) increases over time [43,44]. We reported that the spectrum of mutations in 10 HVR1 positions, which influence physicochemical properties of amino acids, changes as infection progresses from the acute to the chronic stage (Figure 1). A classification model built using these 10 positions distinguished acute from chronic cases with a cross-validation accuracy of 88%, suggesting that mutations occupying these positions may act as signature of acute or chronic infection [45]. Work has begun to determine if these preliminary findings can be confirmed in prospectively obtained patient samples.

**Table 1. Continued**

<table>
<thead>
<tr>
<th>Type of assay</th>
<th>HCV antigens</th>
<th>Genotype</th>
<th>Protein expression system</th>
<th>Manufacturer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘In-house’ improved EIA for IgM detection by NEIBM</td>
<td>Core, NS3, NS4, NS5</td>
<td>NA</td>
<td>NA</td>
<td>GenWay Biotech Inc., San Diego, CA, USA</td>
<td>[16]</td>
</tr>
</tbody>
</table>

**Figure 1. Physicochemical properties of HVR1 positions**

Reproduced with permission from [45]. Mutations in the amino acid (AA) positions (P; 1, 4, 5, 12, 14, 15, 16, 21, 22 and 29) shown along the x-axis may affect their physicochemical properties. The differences between the mean of each property in chronic samples minus the mean of the same property in acute samples are shown along the y-axis. Only significantly different variables (P<0.05) are shown. HVR1, hypervariable region 1.
Other approaches

The role of viral host factors in spontaneous clearance and prediction of treatment outcome, predominantly in polymorphisms in the interleukin (IL)-28B gene, which are associated with HCV clearance, has been investigated in several studies [46–48]. Additionally, it has also been reported that plasma levels of IL-18 increases early in the course of infection, is increased during reinfection but is decreased during persistent infection [49]. Therefore, these ILs could potentially be used as markers of infection and, in combination with viral markers, could aid in the early diagnosis of HCV infection. Finally, the possibility of a new field of research – ‘serolomics’ – has been raised [50]. It anticipates the expansion of diagnostics based on multiplexed analysis of markers of infection present in serum.

Conclusions

It is still not possible to make a diagnosis of recent HCV infection from single-serum testing. Nonetheless, measurement of anti-HCV IgG avidity, IgG titre and identification of signature mutations in the HCV genome are promising. Much more validation work is required to assure that these approaches are sufficiently robust to be widely applicable.

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Disclosure statement

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References


