Hepatitis C Core Antigen Testing: A Reliable, Quick, and Potentially Cost-effective Alternative to Hepatitis C Polymerase Chain Reaction in Diagnosing Acute Hepatitis C Virus Infection

Fiona V. Cresswell,1 Martin Fisher,1,2 Daniel J. Hughes,1 Simon G. Shaw,1 Gary Homer,3 and Mohammed O. Hassan-Ibrahim3

1Lawson Unit, Department of HIV and Sexual Health, Royal Sussex County Hospital, 2Brighton and Sussex Medical School, and 3Department of Virology/Microbiology and Infection, Royal Sussex County Hospital, Brighton, United Kingdom

Hepatitis C virus (HCV) is increasingly common among human immunodeficiency virus (HIV)-infected men who have sex with men. We evaluated the efficacy of HCV core antigen in diagnosing acute HCV in an HIV-infected cohort. Compared with HCV polymerase chain reaction, core antigen proved sensitive (100%) and specific (97.9%). As a quick, simple, and cost-effective test, it has considerable utility in screening for acute HCV.

Keywords. acute hepatitis C; hepatitis C core antigen; PCR; diagnosis.

Outbreaks of acute hepatitis C are being increasingly reported, almost exclusively among human immunodeficiency virus (HIV)-infected men who have sex with men (MSM). The incidence is at least 8-fold greater than among HIV-uninfected MSM [1], which may be driven by an increase in plasma and semen hepatitis C virus (HCV) RNA loads in the presence of HIV along with a resurgence of unsafe sexual practices and injection and other recreational drug use [2].

Less than half of persons acutely infected with HCV experience symptoms and, if present, those symptoms are frequently nonspecific [3]. It is therefore recommended in US, European, and British guidelines that HIV-infected MSM should be screened for HCV at 6-month intervals with serum aminotransferases and at least annually with anti-HCV antibody [4–6]. However, it is well recognized that HCV antibody seroconversion can be delayed in HIV-infected individuals, with only two-thirds testing positive at 3 months and 5% remaining negative up to 1 year after infection [7, 8]. This has significant implications for timely diagnosis, treatment, partner notification, contact tracing, and reduction of onward transmission. Furthermore, individuals who have cleared previous HCV infection remain HCV antibody infected, making it an unhelpful marker for screening for reinfection. As HCV treatment expands with wider availability of all-oral therapy, a cost-effective screening test for reinfection will be increasingly important.

As such, a nucleic acid amplification test for HCV RNA by quantitative reverse transcription polymerase chain reaction (qRT-PCR) is recommended in this group if aminotransferases are elevated or if there has been a high-risk exposure [4–6]. However, HCV qRT-PCR is expensive, time-consuming, and requires advanced technical skills and equipment. A cheaper and quicker assay for initial testing for suspected HCV is needed, particularly in low-resource settings [9].

Less expensive and time-consuming tests against HCV core antigen have recently become available, which may be useful in diagnosing acute infection. HCV core antigen can be used as a marker of viremia, as there is good nonlinear correlation with HCV RNA (r = 0.87 vs Abbot Real-Time qRT-PCR) with the lower limit of detection corresponding to HCV RNA load of 700–1100 IU/mL [10]. This assay is available in Europe, Canada, Australasia, South and Central America, and Asia, but Food and Drug Administration approval has not yet been sought for the United States. To date, only 3 studies have been published in the coinfected population, including only 1 using the Abbot Architect platform further information about previous studies evaluating HCV core antigen can be found in Appendix 1. For this reason, routine use of HCV core antigen testing in either mono- or coinfected patients cannot be currently recommended, and more data are needed [5, 6].

In this study we compare the utility of HCV core antigen compared with qRT-PCR in the diagnosis on acute HCV in an HIV-infected cohort.

METHODS

Subjects were patients attending a dedicated HIV outpatient clinic. Routine liver function tests were performed every 4–6
months in all patients as per British HIV Association monitoring guidelines [11]. Individuals with newly elevated alanine aminotransferase (ALT) levels above the laboratory upper limit of normal (>41 IU/L men; >31 IU/L women) on routine blood testing between April 2012 and December 2013 were screened for HCV infection using (1) HCV core antigen testing (Abbott Architect); (2) HCV RNA testing (Abbott Real-Time qRT-PCR); and (3) HCV antibody testing (Abbott Architect).

Those with chronically elevated and stable ALT for an already established diagnosis (e.g., steatohepatitis) were excluded. As per the manufacturer’s guideline, a cutoff of 10 fmol/L for HCV core antigen positivity with an indeterminate zone between 3 and 10 fmol/L was adopted. Retrospective testing of stored samples determined acute vs chronic HCV infections, the latter being excluded from the study, consistent with the European recommendations for diagnosing acute infection [5].

Statistical analysis using SPSS (version 22) software was performed; multivariate analysis, sensitivity, specificity, and positive and negative predictive value calculations were calculated; and Spearman coefficient was calculated for the correlation between qRT-PCR and HCV core antigen.

RESULTS

One hundred eleven of the 2058 (5.4%) patients on routine blood monitoring were found to have a newly elevated ALT during the 20-month study. The vast majority (105 [94.6%]) were male, 105 (94.6%) were white, and 101 (91%) were MSM. The majority (89 [80%]) were receiving antiretroviral therapy; the HIV RNA load was undetectable (<40 copies/mL) in 84 (75.7%) of the patients, with a CD4+ count of <350 cells/µL in 23 (20.7%), 350–499 cells/µL in 26 (23.4%), and >500 cells/µL in 62 (55.9%) cases.

Fifteen cases of acute HCV were identified by qRT-PCR (HCV RNA load range, 60 950–14 794 746 IU/mL) (Table 1). HCV core antigen testing correctly identified all 15 acute infections, resulting in a sensitivity of 100% (95% confidence interval [CI], 75%–100%). Retrospective testing of stored serum samples identified 3 additional chronic infections (>6 months’ duration), which were not included in subsequent analyses.

There were no false-positive HCV core antigen results. However, 2 “indeterminate” results were obtained (quantitative value, 4.86 and 3.18 fmol/L). The first individual was retested on the same day and did not become positive on retesting with either assay. His sexual and lifestyle history were explored; there were no HCV risk factors, so screening was not repeated during the study as his ALT normalized. The second individual did become qRT-PCR positive on retesting 5 months later when he remained antigen positive (1576 fmol/L) with an HCV RNA level of 694 201 IU/mL and had a positive HCV antibody. It is not clear whether this was a “false-indeterminate” result or whether it was an extremely early infection that failed to amplify on qRT-PCR, as we had no interim samples that could be analyzed. As we are using qRT-PCR as the gold standard in this analysis, we have included that result as a false-indeterminate. Specificity of HCV core antigen is therefore 97.96% (95% CI, 92.1%–99.6%) with a positive predictive value of 88% (95% CI, 62.2%–97.9%) and a negative predictive value of 100% (95% CI, 95.2%–100%).

HCV antibody was positive in 9 of the 15 cases, giving a sensitivity of 60%, with those antibody-negative at the time of acute HCV diagnosis taking up to 112 days to seroconvert.

All 15 acute HCV infections were in MSM and acquired through sexual intercourse; notably, 3 were reinfections, with the men having previously cleared HCV. HCV genotype 1a predominated (n = 11), but genotypes 1b (n = 1), 3a (n = 1), and 4d (n = 2) were also seen (Table 1). No significant associations with age, ethnicity, HIV RNA, or CD4+ count were seen when comparing with the total population of those with abnormal ALT.

The nonlinear correlation between HCV core antigen (fmol/L) and qRT-PCR (IU/mL) in this dataset was extremely good (r = 0.943, P < .001), in keeping with previously reported results for this assay in monoinfected studies [10].

Potential cost savings exist by introducing HCV core antigen testing routinely as a screening tool in place of qRT-PCR. The cost per individual screen would be approximately $85 less ($108 for qRT-PCR vs $23.4 for HCV core antigen including kit, staff, and laboratory extras, although this will vary between laboratories). We calculate that in our cohort (approximately 2200 patients with HIV infection; 111 with newly raised aminotransferases, 96 of whom were HCV negative), over the 20-month study period the potential cost savings would have been $8160 in kits alone. We estimate additional savings in manpower of 14 days—which equates to approximately $3118 (converted from £1949 at a rate of 1.6) by UK costings (based upon 70 minutes of manpower spared per test).

All except 1 of the individuals diagnosed with acute HCV during the study period have subsequently been assessed in a dedicated HIV/HCV coinfection clinic. Three (20%) cleared spontaneously; 7 elected to take early treatment with pegylated interferon and ribavirin for 24–48 weeks (pending rapid virological response), and all of those who completed treatment exhibited a sustained virological response, with an intent-to-treat response rate of 83%.

CONCLUSIONS

In this study, HCV core antigen testing on the Architect platform displayed high sensitivity (100%) and specificity (97.96%) in diagnosing acute HCV in HIV-infected individuals who experience elevated aminotransferases. It would not have missed any infections if used in place of qRT-PCR.
<table>
<thead>
<tr>
<th>Number</th>
<th>Age</th>
<th>Ethnicity</th>
<th>HCV Acquisition Risk</th>
<th>HCV Genotype</th>
<th>ART</th>
<th>CD4 Count, Cells/µL</th>
<th>HIV RNA Load, cp/mL</th>
<th>ALT at First Test, IU/L</th>
<th>HCV cAg</th>
<th>HCV qRT-PCR Level, IU/mL</th>
<th>HCV cAg Level, fmol/L</th>
<th>HCV Ab</th>
<th>Time to Ab Positivity, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>TDF FTC ETV</td>
<td>524</td>
<td>&lt;40</td>
<td>901</td>
<td>Detected</td>
<td>60 950</td>
<td>181.26</td>
<td>Detected</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>Black Caribbean</td>
<td>MSM</td>
<td>1a</td>
<td>Nil</td>
<td>654</td>
<td>3711</td>
<td>145</td>
<td>Detected</td>
<td>175 068</td>
<td>386.16</td>
<td>Detected</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>White</td>
<td>MSM</td>
<td>3</td>
<td>TDF FTC RAL</td>
<td>168</td>
<td>&lt;40</td>
<td>69</td>
<td>Detected</td>
<td>644 269</td>
<td>2820.82</td>
<td>Not detected</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>TDF EFV 3TC</td>
<td>613</td>
<td>&lt;40</td>
<td>90</td>
<td>Detected</td>
<td>1 017 207</td>
<td>2624.27</td>
<td>Detected</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>TDF FTC EFV</td>
<td>969</td>
<td>&lt;40</td>
<td>249</td>
<td>Detected</td>
<td>798 414</td>
<td>15 171.12</td>
<td>Not detected</td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>TDF FTC EFV</td>
<td>369</td>
<td>&lt;40</td>
<td>81</td>
<td>Detected</td>
<td>39 345</td>
<td>112.3</td>
<td>Not detected</td>
<td>112</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>White</td>
<td>MSM</td>
<td>4</td>
<td>TDF FTC EFV</td>
<td>468</td>
<td>56</td>
<td>58</td>
<td>Detected</td>
<td>6 818 724</td>
<td>15 230.33</td>
<td>Not detected</td>
<td>46</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>TDF FTC EFV</td>
<td>611</td>
<td>&lt;40</td>
<td>210</td>
<td>Detected</td>
<td>1 027 129</td>
<td>3172.8</td>
<td>Detected</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>TDF FTC DRV/r</td>
<td>665</td>
<td>&lt;40</td>
<td>1074</td>
<td>Detected</td>
<td>2 994 028</td>
<td>5186.32</td>
<td>Detected</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>34</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>ABC 3TC NVP</td>
<td>592</td>
<td>&lt;40</td>
<td>59</td>
<td>Detected</td>
<td>28 654 482</td>
<td>&gt;20 000</td>
<td>Detected</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>43</td>
<td>White</td>
<td>MSM</td>
<td>1b</td>
<td>TDF ETV DRV/r RAL</td>
<td>951</td>
<td>&lt;40</td>
<td>78</td>
<td>Detected</td>
<td>1 357 494</td>
<td>6271.29</td>
<td>Not detected</td>
<td>62</td>
</tr>
<tr>
<td>12</td>
<td>48</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>TDF FTC DRV/r</td>
<td>126</td>
<td>36 316</td>
<td>665</td>
<td>Detected</td>
<td>6 94 210</td>
<td>1576.38</td>
<td>Detected</td>
<td>NA</td>
</tr>
<tr>
<td>13</td>
<td>42</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>TDF FTC DRV/r</td>
<td>410</td>
<td>&lt;40</td>
<td>1972</td>
<td>Detected</td>
<td>8 418 803</td>
<td>14 198.72</td>
<td>Detected</td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>51</td>
<td>White</td>
<td>MSM</td>
<td>1a</td>
<td>TDF FTC EFV</td>
<td>266</td>
<td>&lt;40</td>
<td>131</td>
<td>Detected</td>
<td>8 882 949</td>
<td>&gt;20 000</td>
<td>Not detected</td>
<td>90</td>
</tr>
<tr>
<td>15</td>
<td>46</td>
<td>White</td>
<td>MSM</td>
<td>4</td>
<td>TDF FTC EFV</td>
<td>602</td>
<td>&lt;40</td>
<td>122</td>
<td>Detected</td>
<td>14 794 746</td>
<td>&gt;20 000</td>
<td>Detected</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: 3TC, lamivudine; ABC, abacavir; ALT, alanine aminotransferase; ART, antiretroviral therapy; cAg, core antigen; DRV/r, darunavir/ritonavir; EFV, efavirenz; ETV, etravirine; FTC, emtricitabine; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MSM, men who have sex with men; NA, not applicable; NVP, nevirapine; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RAL, raltegravir; TDF, tenofovir.
Similar sensitivity (100%) and specificity (97.7%) have been reported in abstract form by another study using the same platform comparing stored serum panels from HIV-infected patients with known acute HCV or chronic HCV, or those negative for HCV [12]. In keeping with existing data in HIV-uninfected individuals, HCV core antigen sensitivity dropped off at low HCV RNA loads (625 IU/mL) [10]. Such low viral loads are rarely seen in HIV/HCV coinfection. Furthermore, in our study 3 reinfections occurred within the time period, an increasingly recognized phenomenon, supporting the utility of antigen testing in detecting acute reinfection. In HIV-negative cohorts, other studies also report sensitivities >90% with the Architect platform [10].

We estimated a potential savings of $8160 in kits and $3118 in manpower during the study period had HCV core antigen been used in place of qRT-PCR. This may be a significant underestimate given our inclusion criteria. If we included all HCV screening tests in addition to those with newly abnormal amino-transferases, as recommended for MSM after significant sexual risk [6], we believe (based upon historical HCV screening throughout the cohort) these savings may increase, in a cohort of 2200, to $18 275 in materials and $6964 in manpower per year.

HCV core antigen results can usually be issued on the same day vs up to a week for qRT-PCR results due to the necessity to batch samples for cost-effectiveness. Such earlier diagnosis would enable earlier commencement of therapy if indicated for the individual, and would facilitate contact tracing and partner notification. We argue therefore that increased use of HCV core antigen testing in preference to qRT-PCR has potentially substantial benefits to the laboratory, clinician, patient, and public health.

HCV core antigen’s utility in low-resource settings, where PCR may be unavailable, would be even more significant [9]. Compared with HCV antibody screening alone, this test considerably shortens the diagnostic window, as the delay to antibody seroconversion previously reported by others after PCR positivity is confirmed here after antigen positivity [7].

The main limitation of our study is the small sample size. Other general test limitations include potential low-level false positives from antigen carryover from the hepatitis C antibody kit if both tests are done on the same instrument and its low sensitivity at low viral loads, as mentioned above.

A larger study with further cost analyses would be valuable. However, in light of our findings and those of Carney et al [12], we believe that national guidelines should now begin to consider HCV core antigen testing as an integral part of the HCV screening algorithm for acute HCV infection.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.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.

Note

Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References