Hepatitis C antigen testing: a reliable alternative for diagnosing acute HCV infection

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Background

- Although ongoing drug use remains a major mode of HCV transmission in the United Kingdom, it is now widely accepted that there is also a developing epidemic of sexually transmitted HCV amongst HIV infected MSM.
- Since 2008 multiple studies of Hepatitis C virus (HCV) amongst HIV positive men who have sex with men (MSM) have been reported, primarily driven by factors such as an increasing in patients and sexual HCV associated with HCV infection and the recognition of sexual modes of practice.
- Early identification of HCV infection is a population health important for both treatment and reduction in onward transmission.
- HCV antigen testing is recommended if HCV positive MSM are screened for HCV at 12-month interval. If raised alanine transaminase (ALT) and/or antibody to HCV are identified.
- A rapid on-site amplification technique (Pakist) for HCV RNA by quantitative reverse transcription polymerase chain reaction (qRT-PCR) is currently recommended as a diagnostic of acute HCV infection.
- It is known that HCV antibody emergence can delay the detection of HCV in infected individuals, sometimes by several months which may have implications for use, diagnosis and treatment.
- Recently tests for HCV core antigens have become available which may be useful alternatives in diagnosing HCV infection with sensitivities of up to 88% and 100% respectively in seroconvert-infected patients (Architect HCV Ag assay)."and are less expensive and time consuming than qRT-PCR.
- However, there is currently insufficient data to recommend their routine use in clinical guidelines in either those with or without infection.
- The HCV Core Department at the Brighton and Sussex University Hospitals NHS Trust (BSUT) manages around 3,000 HIV positive individuals, 70% of whom are MSM and is experiencing a high incidence of acute HCV infection in the cohort.
- Recent HCV Ag testing has become readily available at this service, allowing first assessment at initial qRT-PCR test to be undertaken.

Objective

- To compare HCV Core antigen testing and HCV qRT-PCR in the detection of acute HCV infection in an HIV-infected population

Methods

- In accordance with British HIV association guidelines routine blood testing (including liver function testing) is performed 4-6 monthly in all patients accessing our unit that
- HCV infected individuals who presented with elevated transaminase (ALT) values above the laboratory upper limit of normal (UKN) of 60 at routine blood sampling between April 2013 and December 2013 were screened for HCV infection using:
- HCV core antigen testing (Architect HCV Ag test)
- HCV qRT-PCR (real time Fast PCR test) (Fastag HCV Ag test)
- HCV antibody testing Abbott (HCV Ab test).
- A quantitative cut-off of 10 mU/ml for HCV antigen positivity with an equivocal zone between 5 and 10 mU/ml was used.
- Plasma samples are routinely stored at the unit, subjects with a positive HCV Ag test result have historical samples available for retrospective testing to evaluate clonal HCV infection from this dataset.
- Subjects were HIV negative for testing were investigated for alternative causes of raised ALT and referred as appropriate.
- All acute HIV positive patients were questioned and referred to a co-infection clinic for assessment for acute co-infection treatment according to British guidelines.
- Statistical analysis was undertaken using SAI-S to undertake multivariate analysis, sensitivity, specificity, positive and negative predictive values calculations, and the Spearman’s correlation coefficient was calculated for the correlation between log10 HCV RNA (CT) and HCV Ag.

Results

Table 1: Subjects demographics

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>Gender</th>
<th>Black</th>
<th>MSM</th>
<th>GA</th>
<th>FTA/TP</th>
<th>SFTIA</th>
<th>CD4 count</th>
<th>Virological status</th>
<th>TDF/FTC/EFV</th>
<th>TDF/FTC, ETV</th>
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<td>6</td>
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<td>Yes</td>
<td>2</td>
<td>0</td>
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<tr>
<td>Negative</td>
<td>5</td>
<td>25.6</td>
<td>M</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>&gt;400</td>
<td>Yes</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: HCV-RNA, HCV cAg and HCV sAb tests

- 111 (4.5%) of the 2,585 patients on routine LFT monitoring were found to have an ALT >40 within 2 years. Of these, there had only ever been HCV test results for 83 (75.2%).
- Of these, 17 were identified by HCV qRT-PCR as acute HCV infections (lowest range 49 - 1410) [95%]
- HCV core antigen testing also correctly identified 15 acute infections (admittedly which were excluded from the subsequent analysis).

Table 3: Data on acute HCV patients

- No significant associations with age, ethnicity, HIV or CD4 count strata were seen when comparing with the total population with those with acute ALT test results. Of note, no infections were in men who have sex with man.
- HCV genotype was predominately (n=10) but genotype (n=10), i.e. 4 (n=4), 6 (n=2) were also seen.
- Initially there were 2 HCV core antigen positive patients (results 0.8 and 1.05/mL), both were also negative as HCV qRT-PCR testing.
- The first did not become positive on retesting with alanine aminotransferase.
- This second did become positive on HCV qRT-PCR, but HCV core antigen was not detected.
- Of this 15 patients with acute HCV infection, six were HCV antibody negative, 2 showed only a weak positive signal and one was already positive from a previously confirmed infection.
- The European and British co-infection guidelines currently recommend that HCV core antigen testing in this analysis was high (90%) and 87% respectively with a positive predictive value of 66% and a negative predictive value of 100%.
- In our data the sensitivity of HCV antibody was 80% in acute infection so is likely to miss 20% of acute infections.

Figure 4: Outcomes of acute HCV infections

- The normal correlation between log10 HCV Ag (mU/ml) and log10 HCV qRT-PCR (copies/ml) in this small dataset was moderate (r=44, p=0.01), albeit slightly lower than previously reported for the Abbot assay in more infected studies.
- If subjects (75%) symptomatic for their infection in the peak phase (50%) is undergoing treatment, and 2 subjects (15%) have cleared the virus (90%) with no abnormalities to date.
- The remaining six subjects are on either awaiting assessment, treatment or are ineligible for current therapies.

Conclusions

In our study, the HCV core antigen test has shown excellent sensitivity (96%) and specificity (97%) for detection of acute HCV infection in HIV positive patients with acceptable turnaround times and is compatible with other studies.14 The performance in these subjects is comparable with HCV qRT-PCR and the non-linear correlation between the two assays is moderate but less than previously reported.

HCV qRT-PCR requires a time-consuming multi-step process, the HCV core antigen test is very quick with a reporting time of one hour.

In conclusion, data of HCV core antigen tests around 1.8% of the peak of HCV qRT-PCR and is not considered by sample number.

Using HCV screening alone is accurate for HCV’s likely to miss early or mild infections due to their long window period if in infected individual.

In this study HCV seropositive testing resulted avoiding 40% of acute HCV infections, suggesting that HCV HCV core antigen testing should now be considered the standard of care in HIV infected patients with advanced ART where acute HCV infection needs to be excluded.

References

5. All null-responders associated with age, ethnicity, HIV or CD4 count strata were seen when comparing with the total population with those with acute ALT test results. Of note, no infections were in men who have sex with man.
6. HCV genotype was predominately (n=10) but genotype (n=10), i.e. 4 (n=4), 6 (n=2) were also seen.
7. Initially there were 2 HCV core antigen positive patients (results 0.8 and 1.05/mL), both were also negative as HCV qRT-PCR testing.
8. The first did not become positive on retesting with alanine aminotransferase.
9. This second did become positive on HCV qRT-PCR, but HCV core antigen was not detected.
10. Of this 15 patients with acute HCV infection, six were HCV antibody negative, 2 showed only a weak positive signal and one was already positive from a previously confirmed infection.
11. The normal correlation between log10 HCV Ag (mU/ml) and log10 HCV qRT-PCR (copies/ml) in this small dataset was moderate (r=44, p=0.01), albeit slightly lower than previously reported for the Abbot assay in more infected studies.
12. If subjects (75%) symptomatic for their infection in the peak phase (50%) is undergoing treatment, and 2 subjects (15%) have cleared the virus (90%) with no abnormalities to date.
13. The remaining six subjects are on either awaiting assessment, treatment or are ineligible for current therapies.
14. Using HCV screening alone is accurate for HCV’s likely to miss early or mild infections due to their long window period if in infected individual.
15. In this study HCV seropositive testing resulted avoiding 40% of acute HCV infections, suggesting that HCV HCV core antigen testing should now be considered the standard of care in HIV infected patients with advanced ART where acute HCV infection needs to be excluded.