Epidemiology and diagnosis of acute hepatitis C virus infection

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EVALUATION OF A HEPATITIS C VIRUS (HCV) ANTIGEN ASSAY FOR ROUTINE HCV SCREENING AMONG MEN WHO HAVE SEX WITH MEN INFECTED WITH HIV

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Background
For detection of early HCV infection and reinfection, commercial HCV-RNA tests are available. However, these tests are relatively time-consuming and expensive. A commercially available test that may supplement current screening methods, targets the HCV core protein.

Methods
During five waves of anonymous surveys at the Amsterdam STI clinic between 2009-2012, all HIV-infected MSM (N = 439) were tested for HCV-antibodies (AxSYM HCV 3.0, Abbott), and HCV-RNA (TMA Versant, Siemens). To evaluate the potential value of the ARCHITECT HCV antigen (HCV-Ag) assay (Abbott), all HCV-RNA-positive sera (N = 31) were tested with this assay, as well as two HIV-infected HCV-RNA-negative controls. In addition, all included samples were tested for alanine aminotransferase (ALT).

Results
Among 439 HIV-infected MSM, 31 (7.1%) tested positive for HCV-RNA; the HCV-Ag assay showed concordant positive results for 31/31 (100%). A substantial number of MSM, i.e., 5/31 (16.1%), had detectable HCV-RNA but were HCV-seronegative at the time of screening and were presumed to have been recently infected. Concordant HCV-RNA-negative results were obtained in 57/60 control-samples. Specificity was 95.0% (95% CI: 86.1-99.0). The detection limit was between 3.0 and 3.7 Log$_{10}$ IU/mL, irrespective of HCV genotype/subtype. ALT concentrations were elevated (i.e., >40 U/L) in 9/31 (29.0%) HCV-RNA positive MSM, including 1/5 (20.0%) MSM with recent HCV-infection.

Conclusions
The HCV-Ag assay proved a valuable screening tool for detection of active HCV infection among HIV-infected MSM with and without anti-HCV. Adding ALT to current screening methods would improve case finding marginally. We therefore recommend implementation of routine HCV-Ag screening for populations at risk for HCV-(re)infection.
Since the mid-1990s, hepatitis C virus (HCV) has emerged as a sexually transmitted infection (STI) among men who have sex with men infected with HIV [1,2]. In 2007 the prevalence of HCV among men who have sex with men infected with HIV attending a large STI clinic in Amsterdam reached 17.8% [3]. Since September 2007, men who have sex with men who attended the Amsterdam STI clinic and were infected with HIV, or unaware of their HIV-status, were offered routine testing for HCV antibodies (anti-HCV).

Anti-HCV is detectable several weeks after infection; two studies showed that over 33% of men who have sex with men infected with HIV were still anti-HCV negative three months after the first positive HCV-RNA test [4,5]. The serodiagnostic window, the time between HCV-infection and the detection of anti-HCV, may be prolonged for HIV-infected individuals compared to individuals without HIV-coinfection; (10-13 weeks versus 5-10 weeks, respectively) [6,7]. Due to this serodiagnostic window, a significant proportion of recently acquired HCV infections may be missed when screening for anti-HCV only. Moreover, high rates of HCV reinfection have been reported among men who have sex with men infected with HIV [8,9]. Commercial HCV-RNA assays are available to diagnose such infections, but these are time-consuming and costly. Therefore, there is room for improvement of currently used routine screening methods to detect recently acquired HCV infections, either primary or recurrent.

In many clinical settings, HCV diagnostic tests are performed when alanine aminotransferase (ALT) levels are elevated, or when specific HCV-related risk behaviour is reported. However, risk behaviour is not always disclosed, and ALT levels can remain normal or rapidly normalize even within the serodiagnostic window [8,10]. Conversely, ALT may be elevated as a result of various other reasons, including cART induced hepatotoxicity, alcohol and/or steroid use, and other viral infections that affect the liver [11].

The ARCHITECT HCV antigen (HCV-Ag) assay (Abbott Laboratories, Abbott Park, IL, USA) is a commercially available immunoassay using chemiluminescent microparticle technology for quantitative measurement of HCV core antigen; a structural protein with a highly conserved sequence across all HCV genotypes [12]. Evaluated was whether the HCV-Ag assay could supplement current routine HCV screening methods, using detectable HCV-RNA as the reference test for sensitivity and specificity.

A technical validation was performed to assess sensitivity. The detection limit of the HCV-Ag assay was determined using a set of 16 HCV-RNA positive samples obtained from clinical patients. Samples were selected based on HCV viral load (≥5.0 Log10 IU/mL; COBAS AmpliPrep/COBAS Taqman HCV assay v2.0, Roche Diagnostics, Pleasanton, CA, USA) and HCV genotype diversity. This set reflects the HCV genotypes/subtypes that are the most prevalent in the Netherlands, and consisted of HCV genotype 1a (n=3), 1b (n=3), 2a (n=1), 2k (n=1), 2b (n=2), 3a (n=3), and 4a (n=3). HCV genotyping had been performed by sequencing part of the NS5B
region [13]. Subsequently, each sample was diluted with HCV-negative plasma (final HCV RNA concentrations: 100,000, 10,000, 5,000, 1,000 and 500 IU/ml) and tested for HCV-Ag. All 16 undiluted samples including their 100,000 IU/ml dilutions showed strong HCV-Ag reactivity. Based on the dilution series, the lower limit of detection of the HCV-Ag assay was estimated to be between 3.0 Log10 and 3.7 Log10 IU/mL, irrespective of genotype/subtype. This lower limit of detection is in agreement with the range reported in other studies [14–16]. The HCV-Ag results of the HCV-RNA dilution experiments are shown in table 1.

Between 2009 and 2012, a total of 1,432 men who have sex with men participated in a series of cross-sectional surveys performed at the Amsterdam STI-clinic [17]. Of them, 439 (30.7%) were infected with HIV, of whom 31 (7.1%) were coinfected with HCV, indicated by a positive HCV-RNA test (TMA VERSANT HCV RNA Qualitative Assay; Siemens Healthcare Diagnostics, Tarrytown, NY, USA). For each of the 31 men with HIV-HCV coinfection, two HIV-infected HCV-RNA negative controls were included from the same survey year. All samples had been screened for anti-HCV (AxSYM HCV 3.0; Abbott Laboratories, Abbott Park, IL, USA) with immunoblot confirmation (Chiron RIBA HCV 3.0 SIA; Ortho-Clinical Diagnostics, Raritan, NJ, USA). Sensitivity and specificity were calculated for each assay and for several clinical test combinations. In accordance with the manufacturer’s instructions, specimens with a HCV-Ag concentration level <3.00 fmol/L were considered nonreactive, values 3.00-10.00 fmol/L were considered weakly reactive and values ≥10.00 fmol/L were considered reactive. Statistical software package STATA Intercooled v13.1 was used for data analysis. Confidence intervals were calculated using exact binomial methods.

The HCV-Ag assay showed fully concordant positive results in all 31 HCV-RNA positive sera (range: 10.4 - >20,000 fmol/L; table 2); sensitivity was 100% (95% CI: 88.8-100). Concordant HCV-Ag negative results were obtained in 57/60 HCV-RNA negative controls; 3/60 sera showed weak false HCV-Ag reactivity, resulting in a specificity of detecting HCV viremia of 95.0% (95% CI: 86.1-99.0). For all 6/60 HCV-RNA negative sera from men with resolved HCV infections (i.e., HCV-RNA negative, anti-HCV positive), concordant HCV-Ag negative results were obtained (table 2).

ALT levels were elevated (i.e., >40 U/L) in 9/31 (29.0%) HCV-RNA positive sera, and in 3/61 (4.9%) negative sera (table 2). Recent -primary- HCV infection was presumed when HCV-RNA was detected without the (confirmed) presence of anti-HCV. Recent HCV was observed in 5/31 (16.1%) subjects, only one of whom had mildly elevated ALT (i.e., 63 U/L). So, 4/5 (80.0%) recent HCV infections would have been missed with a test algorithm consisting of anti-HCV and ALT only.

The HCV-Ag assay proved to be a valuable screening tool for HCV infection among men who have sex with men infected with HIV. All 31 HCV infections were detected, including 5 recently
acquired anti-HCV negative HCV infections. Combined anti-HCV and ALT testing, as currently performed in clinical practice, identified all 26 chronic HCV-infections but missed 4/5 (80%) recent HCV-infections, and was thereby clearly inferior to HCV-Ag testing.

As a result of indeterminate HCV-Ag reactivity in men without HCV infection (n=3), the estimated specificity of HCV-Ag detection was 95%. Depending on the background prevalence this may result in a relatively low positive predictive value. The manufacturer advises re-testing of specimens with an indeterminate HCV-Ag test result, but unfortunately no additional serum was available for re-testing in this study. In addition, the sensitivity and specificity for recently acquired infections was hard to determine due to the small sample-size.

A limitation of the present study is that only qualitative but no quantitative HCV-RNA viral loads were determined for our study samples. Based on the range of HCV-Ag concentrations (i.e., 10.4 - >20,000 fmol/L) of the 31 HCV-RNA positive samples, HCV-RNA loads varied between 3.0 Log10 and >6.0 Log10 IU/mL (calculated using Vermehren et al. [18]). The detection limit of the assay used in the present study was estimated to be between 3.0 Log10 and 3.7 Log10 IU/mL. Most evaluations that were previously performed were among HIV-negative subjects, with two exceptions [19,20]. However, these studies did not evaluate the added value of implementing routine HCV-Ag or ALT screening in a high-risk population, such as men who have sex with men infected with HIV. As several studies have reported on the benefits of HCV-Ag testing in the detection of chronic HCV infection, future studies should focus on the added value of HCV-Ag detection in recently acquired HCV-infections and reinfections [15,18,21,22].

Routine screening for HCV using anti-HCV and ALT testing has been recommended among men who have sex with men infected with HIV [23]. Due to prolonged HCV seroconversion intervals in patients infected with HIV and the low specificity of ALT testing with regards to recent HCV infection, a large proportion of cases with recent HCV infections will be missed (4/5 in our study). Detection of recent HCV infection is important, not only to prevent further transmission, but also to improve treatment success by starting treatment early after infection [24]. Therefore, implementation of routine HCV-Ag screening for populations at risk for HCV-infection is recommended. Screening for HCV-Ag could be used as a cost-saving approach for the detection of recently acquired HCV-infections among risk groups. Anti-HCV testing can be performed to confirm seroconversion in cases with no history of HCV, or to differentiate between acute versus chronic HCV infection. This assay may be of particular benefit to identify HCV-reinfections, which is of ongoing concern in this population, especially in the upcoming era of effective direct acting antiviral therapy.
ACKNOWLEDGMENTS

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The medical ethics committee of the Academic Medical Center (MEC AMC) approved the parent study. No further ethical approval was needed as blinded laboratory samples were used for this study.
### Table 1: HCV Antigenemia among 93 HIV-infected MSM Attending a Large STI Outpatient Clinic in Amsterdam, 2009-2012.

<table>
<thead>
<tr>
<th></th>
<th>HCV-RNA Detectable (N=31)</th>
<th>HCV-RNA Not Detectable (N=62)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recent HCV (n=5)</td>
<td>Chronic HCV (n=26)</td>
<td>Resolved HCV (n=6)</td>
<td>No HCV (n=56)</td>
</tr>
<tr>
<td>HCV-Ag ≥10.00 fmol/L (reactive)</td>
<td>5 (100)</td>
<td>26 (100)</td>
<td>0 (0)</td>
<td>0/54 (0.0)*</td>
</tr>
<tr>
<td>HCV-Ag ≥3.00 fmol/L (at least weakly reactive)</td>
<td>5 (100)</td>
<td>26 (100)</td>
<td>0 (0)</td>
<td>3/54 (5.6)*</td>
</tr>
<tr>
<td>ALT elevated (&gt;40 U/L)</td>
<td>1 (20.0)</td>
<td>8 (30.7)</td>
<td>1 (16.7)</td>
<td>2/55 (3.6)**</td>
</tr>
<tr>
<td>HCV-Ab positive</td>
<td>0 (0)</td>
<td>26 (100)</td>
<td>6 (100)</td>
<td>0/56 (0)</td>
</tr>
<tr>
<td>HCV-Ab positive and ALT elevated, combined</td>
<td>1 (20.0)</td>
<td>26 (100)</td>
<td>6 (100)</td>
<td>2/55 (3.6)**</td>
</tr>
<tr>
<td>HCV-Ab positive and HCV-Ag weakly reactive, combined</td>
<td>5 (100)</td>
<td>26 (100)</td>
<td>6 (100)</td>
<td>3/54 (5.6)*</td>
</tr>
</tbody>
</table>

Numbers are n (%); recent HCV: HCV-Ab negative & HCV-RNA positive; chronic HCV: HCV-Ab positive & HCV-RNA positive; resolved HCV: HCV-Ab positive & HCV-RNA negative. *two samples gave an internal error in HCV-Ag test and were left out of the calculation; **one of these two samples also gave an internal error in the ALT test and was left out of the calculation.
Table 2 HCV-Ag assay results of 16 samples in a dilution experiment. Samples were derived from HCV-positive blood donors in the Amsterdam area, 2013.

NOTE: HCV genotyping/subtyping was performed by sequencing part of the NS5B region. Black=reactive, grey=weakly reactive, white=non-reactive.
REFERENCES


