Journal of Virological Methods 213 (2015) 147–150

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Short communication

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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Article history:
Received 19 August 2014
Received in revised form
19 November 2014
Accepted 25 November 2014
Available online 18 December 2014

Keywords:
Hepatitis C Virus
Acute HCV infection
HCV core antigen
Men who have sex with men
HIV coinfection

A B S T R A C T

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 Log10 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 (Van de Laar et al., 2007; Van der Helm et al., 2011). 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% (Urbanus et al., 2009). 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 3 months after the first positive HCV-RNA test (Thomson et al., 2009; Vanhommerig et al., 2014). 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) (Glynn et al., 2005; Netski et al., 2005). Due to this serodiagnostic window,

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http://dx.doi.org/10.1016/j.jviromet.2014.11.026
0166-0934/

Table 1
HCV antigen assay results of 16 samples in a dilution experiment. Black = reactive, gray = weakly reactive, white = non-reactive. HCV-RNA viral loads of the undiluted samples varied between 5.03 Log^{10} and 7.08 Log^{10} IU/mL; mean was 5.96 Log^{10} IU/mL.

Sample	HCV subtype	HCV viral load (IU/mL)					
		Undiluted	5.0 Log^{10}	4.0 Log^{10}	3.7 Log^{10}	3.0 Log^{10}	2.7 Log^{10}
1	1a						
2	1a						
3	1a						
4	1b						
5	1b						
6	1b						
7	2a						
8	2k						
9	2b						
10	2b						
11	3a						
12	3a						
13	3a						
14	4a						
15	4a						
16	4a						

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 (Lambers et al., 2011; Martin et al., 2013). 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 behavior is reported. However, risk behavior is not always disclosed, and ALT levels can remain normal or rapidly normalize even within the serodiagnostic window (Vogel et al., 2009; Lambers et al., 2011). 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 (Sulkowski, 2004).

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 (McLauchlan, 2000). 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 (Murphy et al., 2007). Subsequently, each sample was diluted with HCV-negative plasma (final HCV RNA concentrations: 100,000, 10,000, 5000, 1000 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 Log^{10} and 3.7 Log^{10} IU/mL, irrespective of genotype/subtype. This lower limit of detection is in agreement with the range reported in other studies (Hosseini-Moghaddam et al., 2012; Ottiger et al., 2013; Park et al., 2010). The HCV-Ag results of the HCV-RNA dilution experiments are shown in Table 1.

Between 2009 and 2012, a total of 1432 men who have sex with men participated in a series of cross-sectional surveys performed at the Amsterdam STI-clinic (Urbanus et al., 2014). 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 to >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 five recently