Published in final edited form as:

AIDS. 2009 January 2; 23(1): 89–93. doi:10.1097/QAD.0b013e32831940a3.

Delayed anti-HCV antibody response in HIV-positive men acutely infected with HCV

Emma C. Thomson, Eleni Nastouli, Janice Main, Peter Karayiannis, Joseph Eliahoo, David Muir, and Myra O. McClure

Department of Virology, Wright-Fleming Institute, Imperial College, London, UK.

Abstract

Objective: An epidemic of acute hepatitis C virus (HCV) infection among HIV-positive men who have sex with men is occurring in urban centers in Western Europe and the United States. Early diagnosis and treatment of HCV results in improved sustained virological response rates. This study compared the sensitivity of reverse transcriptase PCR (RT-PCR) versus antibody screening for the diagnosis of early HCV infection in HIV-positive patients and estimated the length of time from HCV infection to the development of anti-HCV antibodies.

Design: Patients from the St Mary's Acute Hepatitis C Cohort (SMACC) were recruited retrospectively and prospectively between 2004 and 2008.

Methods: Archived plasma samples, obtained at 1–3 monthly intervals for routine monitoring of HIV viral load were assayed retrospectively for HCV in order to assess the sensitivity of RT-PCR and enzyme-linked immunosorbent assay (ELISA).

Results: Forty-three HIV-positive patients with early HCV infection were identified. The median CD4 cell count was 570 cells/ μ l. The median alanine transaminase at the time of the first positive HCV PCR was 65 IU/ml. At this time, 75% of patients had a negative HCV antibody test. Three months later, 37% of patients still had a negative result. After 9 months, 10% of patients had a negative test and 5% remained negative after 1 year.

Conclusion/discussion: Delayed seroconversion in HIV-positive individuals with acute HCV may result in delayed diagnosis and treatment. Where there is a clinical suspicion of recent HCV infection, for example, elevated alanine transaminase levels, HIV-infected patients should be screened for HCV RNA by RT-PCR.

Keywords

acute hepatitis C; antibody; HIV

Introduction

An epidemic of acute hepatitis C infection in HIV-positive men who have sex with men (MSM) has been reported in the UK, France, the Netherlands, Germany and the United States [1–4]. Early diagnosis and treatment of HIV-infected patients with pegylated interferon alpha and ribavirin results in improved sustained virological response (SVR) rates (59% in acutely infected patients versus 40% in chronically infected patients) [1,5], but does not reach the 98% treatment success rate reported in HIV-negative individuals [6]. Antibody

testing is the main screening method for HCV infection in HIV-infected individuals in the UK [7]. However, serological methods in HIV-infected patients might not be the optimal screening method for early HCV diagnosis [8]. The objectives of this study were to assess the sensitivity of serology versus reverse transcriptase PCR (RT-PCR) performed on blood samples collected at 1–3 monthly intervals in 43 HIV-positive patients presenting with acute HCV infection, and to estimate the median time from PCR amplification of HCV sequences to the development of antibodies.

Methods

Case definition: patients were diagnosed with acute HCV if they had a negative RT-PCR within 8 months of the first positive RT-PCR test, or a preceding negative anti-HCV antibody within 6 months of the first positive RT-PCR, if HIV-negative at this time (two coinfecting patients). Spontaneous clearance was defined as a negative PCR test 6 months after the last positive PCR.

Stored plasma samples collected at 1–3 monthly intervals from 43 HIV-positive patients diagnosed with HCV infection were available for retrospective analysis. The median duration of follow-up was 962 days [interquartile range (IQR) 567–1347 days]. Samples were tested for HCV RNA by qualitative nested RT-PCR, designed to amplify the 5' untranslated region (outer primers HCV-26 5'-GTCTAGCCATGGCGTTAG-3', HCV-27 5'-GCACGGTCTACGAGACCT-3', inner primers HCV-28 5'-GTGTCGTGCAGCCTCCAG-3' and HCV-29 5'-GGGGCACTCGCAAGCACC-3', limit of detection <10 copies/ml). Quantitative HCV viral loads were measured by real-time PCR (Abbott, limit of detection <12 IU/ml). Antibodies to HCV core, NS3 and NS4 proteins were assayed by a third-generation enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (Monolisa Anti-HCV Plus, BioRad, Kent, UK). Seroconversion time was calculated as the time between the first positive PCR and first positive antibody test. A further analysis of seroconversion time was carried out using samples from a subgroup of patients, who had samples taken at 4-weekly intervals.

Mann–Whitney tests and Spearman's rank correlation were carried out using STATA 10.0 software.

Ethical approval for the study was granted by the Riverside Research Ethics Committee, Charing Cross Hospital, London (05/Q0401/17).

Results

Forty-three MSM with acute HCV infection were identified. These patients constitute part of an emerging outbreak of HCV in HIV-positive men presenting to St Mary's Hospital, London. The risk factors for infection were intravenous, intramuscular, intranasal and intrarectal drug use (80%) and recent unprotected anal intercourse (94%). Fifty-three percent of patients were receiving treatment HAART at the time of infection. The median time from the last negative to the first positive RT-PCR was 96 days (IQR 70–119 days).

The median HCV viral load was 1 836 595 IU/ml. A variety of HCV genotypes were identified. Genotype 1a was the most prevalent (75%), genotype 4d occurred in 17.5% and genotypes 1b and 3a in 2.5%, respectively. One patient was coinfecting with genotypes 1a and 4d.

The median CD4 cell count at the time of HCV acquisition was 570 cells/ μ l (Table 1). Patients who cleared infection spontaneously had a higher median CD4 cell count of 830 cells/ μ l than those who progressed to chronic infection (median CD4 cell count of 540 cells/

μl ; $P=0.09$). The median nadir CD4 cell count was 325 cells/ μl . Those patients who spontaneously cleared infection also had a higher nadir CD4 cell count (median 440 cells/ μl versus median 320 cells/ μl ; $P=0.07$). No correlation between CD4 cell count at the time of HCV diagnosis and seroconversion time was found ($P=0.96$). However, a weak negative association between nadir CD4 cell count and seroconversion time was observed ($P=0.09$).

The sensitivity of HCV PCR, antibody and alanine transaminase (ALT) was assessed at 1–3 monthly time-points. The median ALT at baseline was 65 IU/ml (normal <40 IU/ml). Elevated ALT levels were present in 76% of patients at baseline and 74% at 3 months. Most patients (88%) had an elevated ALT at either baseline or 3 months postinfection. An elevated ALT level was significantly more sensitive than antibody testing ($P=0.017$). When a lower normal value of less than 30 IU/ml was used (recently proposed as normal in men [9]), the sensitivity of ALT testing increased to 79% at baseline and 82% at 3 months. As many as 93% of patients had an ALT level higher than 30 IU/ml at either baseline or 3 months postinfection. The ALT levels at baseline and at 3 months postinfection were not significantly different in patients who cleared infection spontaneously from those who progressed to chronicity ($P=0.66$ and 0.92 , respectively).

On first amplification of HCV, only 25% of patients were serologically positive. Antibody detection rose from 63% by 3 months to 87% at 6 months, 90% at 9 months and 95% by 12 months (Fig. 1).

The median time from HCV RNA detection by PCR to the development of anti-HCV antibodies was estimated to be 91 days (range 0–1206 days). An analysis of seroconversion time in a subset of patients where the last negative and first positive RT-PCR were less than 30 days apart ($n=8$) revealed a median seroconversion time of 158 days (range 47–209 days). Two patients (4.7%) developed anti-HCV antibodies more than 1 year after the initial infection, one of whom did not become antibody positive until after 3 years. Seroconversion in one of these patients was associated with a superinfection with genotype 1a virus on a background of genotype 4 infection.

Seroconversion time in ‘spontaneous clearers’ and ‘progressors’ was not significantly different ($P=0.88$). However, seroconversion time was significantly longer in patients with a low HCV viral load ($P=0.04$) or low ALT ($P=0.0005$) at the time of the first positive PCR. A low ALT level at 3 months into HCV infection was also weakly associated with prolonged seroconversion time ($P=0.08$).

The early development of anti-HCV antibodies did not correlate with patient age ($P=0.78$), HCV genotype ($P=0.51$) or HIV viral load ($P=0.22$).

Discussion

Early treatment for acute HCV improves outcome in HIV-positive patients [1,5]. Tests available for the diagnosis of HCV infection in the acute phase vary in sensitivity. Third-generation antibody ELISA tests detect multiple antigenic determinants (core, NS3, NS4, NS5) and have been developed to increase sensitivity. HIV-negative patients generally produce an antibody to HCV within 6 weeks of infection [10]. We have shown in this study that in HIV-positive individuals, in contrast, screening with a third-generation antibody assay in the first 6 months of infection is inadequate in most patients. Furthermore, at 1 year, nearly 5% of patients remain seronegative. The use of a combined antigen-antibody test in HIV-positive individuals could increase screening sensitivity but may not be cost-effective and is not as sensitive as PCR [11]. This delay in the formation of antibodies may result in significant delay in diagnosis of HCV in HIV-positive individuals and patients may lose the opportunity to have their infection treated successfully (59% success rate versus 40%) [1,5].

An accurate estimate of seroconversion time (time of infection to the development of antibodies) in this group of patients is limited by the 3 monthly sampling window, and therefore, a subgroup of eight patients who had been sampled at monthly intervals (<30 days) were identified, allowing a more precise assessment of seroconversion time. In this group, the median time from infection to the development of antibodies was estimated as 158 days (IQR 47–209 days).

Factors found to correlate with a prolonged seroconversion time included low ALT level and low HCV viral load at baseline, perhaps reflecting reduced B cell recognition and lower antigenic load, respectively. The CD4 cell count at the time of HCV infection was not associated with seroconversion time, although patients with a lower nadir CD4 cell count had a slightly increased likelihood of a delayed antibody response, suggesting that prolonged CD4 dysfunction may occur, despite an increased CD4 cell count following HAART. Patients with a lower CD4 cell count at the time of infection were less likely to clear infection with HCV spontaneously (540 versus 830 cells/ μ l). A lower nadir CD4 cell count was also weakly associated with a lower chance of clearing HCV (320 versus 440 cells/ μ l). However, seroconversion time was not significantly associated with clearance ($P=0.88$).

Other immunosuppressed patient groups, such as solid-organ transplant and hemodialysis recipients have previously exhibited delayed anti-HCV antibody responses [12–14]. The phenomenon has also been described previously in a case series of three immuno-suppressed HIV-infected individuals (CD4 cell counts 14–266 cells/ μ l) [15], with formation of anti-HCV antibodies following treatment with HAART and resultant immune restoration. This phenomenon was not observed in our cohort of patients, perhaps because of the higher median CD4 cell count (570 cells/ μ l) in this patient population. Thus, delayed anti-HCV seroconversion may also occur frequently in HIV-positive patients who have normal CD4 cell counts.

The association between nadir CD4 cell count and delayed seroconversion time, as well as likelihood of clearing HCV spontaneously, is likely to reflect impaired CD4 function, despite the restoration of absolute counts.

Conclusion

We conclude that elevated ALT levels in at-risk HIV-positive patients should indicate testing for HCV by PCR in order to diagnose infection early. Furthermore, HIV-infected patients diagnosed with acute HCV should have a preceding negative HCV RNA test (performed retrospectively) to accurately establish the timing of infection.

Acknowledgments

This study was funded by the Wellcome Trust and the Mason Medical Foundation. Antibody detection kits were donated free of charge by BioRad.

Emma Thomson and Eleni Nastouli carried out the experimental work. Peter Karayiannis, Myra McClure and David Muir supervised the project. Janice Main provided clinical expertise. Joseph Eliahoo provided statistical support. All authors contributed to the final version of the paper.

References

1. Gilleece YC, Browne RE, Asboe D, Atkins M, Mandalia S, Bower M, et al. Transmission of hepatitis C virus among HIV-positive homosexual men and response to a 24-week course of pegylated interferon and ribavirin. *J Acquir Immune Defic Syndr*. 2005; 40:41–46. [PubMed: 16123680]

2. Gambotti L, Batisse D, Colin-de-Verdiere N, Delaroque-Astagneau E, Desenclos JC, Dominguez S, et al. Acute hepatitis C collaborating group. Acute hepatitis C infection in HIV-positive men who have sex with men in Paris, France, 2001–2004. *Euro Surveill.* 2005; 10:115–117. [PubMed: 16077209]
3. Vogel M, Bieniek B, Jessen H, Schewe CK, Hoffmann C, Baumgarten A, et al. Treatment of acute hepatitis C infection in HIV-infected patients: a retrospective analysis of eleven cases. *J Viral Hepat.* 2005; 12:207–211. [PubMed: 15720537]
4. Fierer DS, Uriel AJ, Carriero DC, Klepper A, Dieterich DT, Mullen MP, et al. Liver fibrosis during an outbreak of acute hepatitis C virus infection in HIV-infected men: a prospective cohort study. *J Infect Dis.* 2008; 198:683–686. [PubMed: 18627270]
5. Torriani FJ, Rodriguez-Torres M, Rockstroh JK, Lissen E, Gonzalez-Garcia J, Lazzarin A, et al. Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N Engl J Med.* 2004; 351:438–450. [PubMed: 15282351]
6. Jaekel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M, et al. Treatment of acute hepatitis C with interferon alfa-2b. *N Engl J Med.* 2001; 345:1452–1457. [PubMed: 11794193]
7. Nelson M, Matthews G, Brook MG, Main J, BHIVA Coinfection Guidelines Committee. British HIV Association guidelines on HIV and chronic hepatitis: coinfection with HIV and hepatitis C virus infection (2005). *HIV Med.* 2005; 6(Suppl 2):96–106. [PubMed: 16011539]
8. Chamie G, Bonacini M, Bangsberg DR, Stapleton JT, Hall C, Overton ET, et al. Factors associated with seronegative chronic hepatitis C virus infection in HIV infection. *Clin Infect Dis.* 2007; 44:577–583. [PubMed: 17243063]
9. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med.* 2002; 137:1–10. [PubMed: 12093239]
10. Netski DM, Mosbrugger T, Depla E, Maertens G, Ray SC, Hamilton RG, et al. Humoral immune response in acute hepatitis C virus infection. *Clin Infect Dis.* 2005; 41:667–675. [PubMed: 16080089]
11. Schnuriger A, Dominguez S, Valantin MA, Tubiana R, Duvivier C, Ghosn J, et al. Early detection of hepatitis C virus infection by use of a new combined antigen-antibody detection assay: potential use for high-risk individuals. *J Clin Microbiol.* 2006; 44:1561–1563. [PubMed: 16597894]
12. David-Neto E, Abdallah KA, Bassit L, Pereira AN, Alquezar AS, Fonseca JA, et al. Anti-HCV antibody is sensitive but not sufficient to detect HCV active infection in renal transplanted patients: the role of PCR for HCV-RNA. *Transplant Proc.* 1997; 29(1–2):781–782. [PubMed: 9123523]
13. Schroeter M, Zoellner B, Polywka S, Laufs R, Feucht HH. Prolonged time until seroconversion among hemodialysis patients: the need for HCV PCR. *Intervirology.* 2005; 48:213–215. [PubMed: 15920344]
14. Preiksaitis JK, Cockfield SM, Fenton JM, Burton NI, Chui LW. Serologic responses to hepatitis C virus in solid organ transplant recipients. *Transplantation.* 1997; 64:1775–1780. [PubMed: 9422419]
15. John M, Flexman J, French MA. Hepatitis C virus-associated hepatitis following treatment of HIV-infected patients with HIV protease inhibitors: an immune restoration disease? *AIDS.* 1998; 12:2289–2293. [PubMed: 9863871]

Fig. 1. Time to seroconversion

Seroconversion time (days) was calculated as the time from the first PCR positive sample to the first positive antibody (range 0–1206 days). Four patients (patients 30, 36, 39 and 40 shown in white) did not produce an antibody by 133, 183, 205 and 218 days of follow-up. Seven patients (asterisked) spontaneously cleared the infection, two of whom did not produce an antibody response.

Table 1

Patient characteristics.

	All	Clearers	Progressors	P	HAART	No HAART	P
Number of patients	43	7 (1 6%)	36 (84%)	–	23 (53%)	20 (47%)	–
Median age (years)	37 (34–41)	35 (27–44)	38 (33–40)	0.72	39 (35–44)	35 (33–40)	0.12
Median CD4 cell count (cells/ μ l)	570 (415–830)	830 (530–1000)	540 (407–815)	0.09	735 (455–893)	510 (380–720)	0.04
Median nadir CD4 cell count (cells/ μ l)	325 (208–418)	440 (230–830)	320 (150–395)	0.07	270 (70–340)	380 (310–455)	0.009
Median HIV VL (copies/ml)	913 (50–23 662)	460 (250–53 579)	2537 (50–20 856)	0.45	<50 (<50–317)	14 397 (2699–83 287)	0.0001
Median HCV VL (IU/ml)	1 836 595 (84 377–8 947 398)	22 801 922 (46.5–77 042 483)	1 386 990 (141 709–7 692 310)	0.58	585 071 (30 778–8 529 035)	2 790 210 (927 653–10 012 463)	0.21
Seroconversion time (days)	91 (63–151)	118 (45–196)	90.5 (63–151)	0.88	105 (63–183)	90.5 (61–133)	0.49
ALT peak (IU/ml)	332 (154–849)	340 (126–1080)	326 (175–664)	0.98	338 (194–816)	326 (126–946)	0.8
Median ALT at 0 month (IU/ml)	65 (41–152)	70 (16–93)	62 (41–155)	0.66	46 (32–147)	81 (39–154)	0.31
Percentage elevated ALT	76	66	77		76	75	
Median ALT at 3 months (IU/ml)	133 (37–319)	93 (21–1006)	140 (39–280)	0.92	87 (27–322)	155 (46–311)	0.34
Percentage elevated ALT	74	71	75		82	68	

Patient baseline characteristics; age, CD4 cell count, HIV viral load, treatment with HAART, HCV viral load (VL) and alanine transaminase (ALT) are shown. The interquartile range for each variable is shown in parentheses.