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

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Background

- Although injecting 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[1]
- Since 2004 multiple outbreaks of Hepatitis C virus (HCV) amongst HIV-positive men who have sex with men (MSM) have been reported, possibly driven by factors such as an increase in plasma and semen HCV viral loads with HIV co-infection and the resurgence of unsafe sexual practices
- Early identification of HCV infection in this population has important implications for both treatment and reduction of onward transmission
- European and British co-infection guidelines currently recommend that HIV-positive MSM be screened for HCV at 6-month intervals if raised alanine transaminase (ALT) and annually for HCV antibodies [2,3]
- A nucleic acid amplification technique (NAAT) for HCV RNA by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) is currently recommended if a diagnosis of acute HCV is suspected
- It is known that HCV antibody seroconversion can be delayed in HIV-infected individuals, sometimes by several months[4] which may have implications for timely diagnosis and treatment
- Recently tests for HCV core antigen have become available which may be useful alternatives in diagnosing HCV infection with sensitivities and specificities as high as 99.7% and 100% respectively in mono-infected patients (Architect HCV Ag assay, Abbott), [5] 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 mono or co-infected patients* [2]
- The HIV Outpatient Department at the Brighton and Sussex University Hospitals NHS Trust (UK) manages around 2,146 HIV positive individuals, 78% of whom are MSM and is experiencing a high incidence of acute HCV infections in this cohort
- Recently HCV Ag testing has become routinely available at this service, allowing its' performance against qRT-PCR 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 monitoring guidelines[6] routine blood testing (including liver function testing) is performed 4-6 monthly in all patients accessing care at this unit
- HIV-infected individuals who presented with alanine transaminase (ALT) values above the laboratory upper limit of normal (ULN) of 41 IU at routine blood sampling between April 2012 and December 2013 were screened for HCV infection using:
  - HCV core antigen testing (Abbott Architect)*
  - HCV RNA test (Abbott Real-Time qRT-PCR)*
  - HCV antibody testing (Abbott Architect +/- BioRad)*
- A quantitative cut-off of 10 fmol/L for HCV antigen positivity with an equivocal zone between 3 and 10 fmol/L was used
- Plasma samples are routinely stored at this unit; subjects with a positive HCV Ag test result had historical samples available for retrospective testing to exclude chronic HCV infections from this dataset
- Subjects who were HCV negative on testing were investigated for alternative causes of raised ALT and referred as appropriate
- All acute HCV positive patients were genotyped and referred to a co-infection clinic for assessment for acute anti-HCV treatment according to British guidelines [2]
- Statistical analysis was undertaken using SAS 9.4 to undertake multivariate analysis, sensitivity, specificity, positive and negative predictive value calculations, and the Spearman coefficient [which was calculated for the correlation between log10(qRT-PCR) and log10(qHCV Ag)]

*as per manufacturers recommendations

Results

Table 1: Subjects demographics

Age (years)	41	(27-73)
Gender		
Male	105	(94.6 %)
Female	6	(5.4%)
Ethnicity		
White	105	(94.6%)
Black	3	(2.7%)
Asian	3	(2.7%)
Route of HIV acquisition		
IVDU	4	(3.6%)
MSM	101	(91%)
Heterosexual	6	(5.4%)
HIV parameters		
viral load (copies/mL)		
undetectable	89	80.2%
<1000	4	3.6%
1000-10 000	9	8.1%
>10 000	9	8.1%
CD4		
<100	0	0%
100-199	4	4.3%
200-349	19	17.1%
350-499	26	23.4%
>500	62	55.9%
On ART therapy		
Yes	89	81%
No	22	19%

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

			HCV core antigen			HCV antibody		
			positive	indeterminate	negative	positive	Weak positive	negative
HCV-RNA	Detectable	15	15	0	0	7	2	6
	Undetectable	97*	0	2	95	2	1	94

- 111 (5.4%) of the 2058 patients on routine LFT monitoring were found to have an ALT >41 (men) or >31 (women). Six of these had only mild rises in ALT of <2.5X ULN)
- 15 were identified by HCV qRT-PCR as acute HCV infections (viral load range 60 950 – 14 794 746)
- HCV core antigen testing also correctly identified all 15 acute infections
- Retrospective testing of stored serum samples identified three chronic infections (>6months) which were excluded form the subsequent analyses

Table 3: Data on Acute HCV patients

Subject	Age	Ethnicity	Route of HCV acquisition	HCV Geno-type	Pre-existing liver pathology	ART	CD4 count at first testing	HV Viral load at first testing	ALT at first testing	HVC PCR	HCV PCR	HCV cAg	HCV sAb	Time to Ab positivity (days)
1	47	White	MSM	1a	EtOH excess	TDF/FTC, ETV	524	<40	901	Detected	60950	181.26	weak reactive	n/a
2	45	Black - Caribbean	MSM/IVDU	1a	-	-	654	3711	145	Detected	175068	386.16	Detected	13
3	45	White	MSM	3	-	TDF/FTC, RAL	168	<40	69	Detected	644269	2820.82	Not Detected	6
4	60	White	MSM	1a	Previous HCV	TDF,EFV,3TC	613	<40	90	Detected	1017207	2624.27	Detected	n/a
5	38	White	MSM	1a	Previous HCV	TDF/FTC/EFV	969	<40	249	Detected	798414	15171.12	Not detected	42
6	50	White	MSM	1a	-	TDF/FTC, DRV/r	369	<40	81	Detected	1530083	112.3	Not Detected	112
7	31	White	MSM	4	-	TDF/FTC/EFV	468	56	58	Detected	6818724	15230.33	Not Detected	46
8	48	White	MSM	1a	-	TDF/FTC/EFV	611	<40	210	Detected	1027129	3172.8	Detected	n/a
9	50	White	MSM	1a	-	TDF/FTC, DRV/r	665	<40	1074	Detected	2994028	5186.32	Detected	n/a
10	34	White	MSM	1b	EtOH excess	ABC/3TC, NVP	592	<40	59	Detected	28654482	>20000	very weak reactive	n/a
11	43	White	MSM	1b	-	TDF,ETV,DRV/r, RAL	951	<40	78	Detected	1357494	6271.29	Not Detected	62
12	48	White	MSM	1a	-	TDF/FTC/DRV/r	126	36316	665	Detected	694210	1,576.38	Detected	n/a
13	42	White	MSM	1a	-	TDF/FTC, DRV/r	410	<40	1972	Detected	8418803	14,198.72	Detected	n/a
14	51	White	MSM	1a	-	TDF/FTC/EFV	266	<40	131	Detected	2962900	20,000.00	Not Detected	90
15	46	White	MSM	4	-	TDF/FTC/EFV	602	<40	122	Detected	14794746	8745.01	Detected	n/a

- No significant associations with age, ethnicity, HIV VL or CD4 count strata were seen when comparing with the total population with those with abnormal ALT test results. Of note, all infections were in men who have sex with men
- HCV genotype 1a predominated (n=11) but genotypes 1b (n=1), 3a (n=1), 4d (n=2) were also seen
- Initially there were 2 HCV core antigen 'indeterminate' results (4.86 and 3.18 fmol/L), both were also negative on HCV qRT-PCR testing
  - The first did not become positive on retesting with either assay.
  - The second did become positive on HCV qRT-PCR and HCV core antigen 5 months later.
- Of the 15 patients with acute HCV infection, six were HCV antibody negative, 2 showed only a 'weakly positive' signal and one was already positive from a previously cleared infection
- The sensitivity and specificity of HCV core antigen testing in this analysis was high (100% and 97.96% respectively) with a positive predictive value of 88% and a negative predictive value of 100%
- In contrast the sensitivity of HCV antibody was 60% in early infection so is likely to miss 40% of acute infections

Figure 4: Outcomes of acute HCV infections

Outcome	Number
Cleared Spontaneously	2
Not eligible for treatment	2
Awaiting assessment	1
Awaiting treatment	3
On treatment	5
Treated and cleared	2

- The non-linear correlation between log10(HCV Ag) (fmol/L) and log10(HCV qRT-PCR) (cp/mL) in this small dataset was moderate (r= 0.60, p<0.05), albeit slightly lower than previously reported for the Abbot assay in mono-infected studies [5,7]
- 2 subjects (13%) spontaneously cleared their infection in the acute phase, 5 (33%) are undergoing treatment, and 2 subjects (13%) have cleared the virus (SVR12) with treatment to date
- The remaining six subjects are either awaiting assessment, treatment or are ineligible for current therapies

Conclusions

- In our study the HCV core antigen test has shown excellent sensitivity (100%) and specificity (97.96%) for detection of acute HCV infection in HIV positive patients with elevated transaminase levels and is comparable with other studies [5, 8]
- It's performance in these subjects is comparable with HCV qRT-PCR and the non-linear correlation between the two assays is moderate but lower than previously reported
- Whereas 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
- The current HCV RNA test (qRT-PCR) requires a minimum batch sample size of 32 samples for cost minimisation
- In contrast the Abbot HCV core antigen test costs around 1/5th of the price of HCV qRT-PCR and is not cost-constrained by sample number
- Using HCV serology alone to screen for acute HCV is likely to miss many early infections due to its long window period in HIV infected individuals.
- In this study HCV serological testing missed approximately 40% of acute HCV infections, suggesting that HCV PCR or HCV antigen testing should now be considered the standard of care in HIV-infected patients with abnormal LFTs in who acute HCV infection needs to be excluded

References

1.Feirer, D.S. Curr. Infect. Dis. Report (2010),12, (2) 118-25 Epidemic of Sexually Transmitted Hepatitis C Virus Infection Among HIV-Infected Men

2. Rockstroh R, Acute HCV in HIV infected individuals. Recommendations from the European AIDS Treatment Network (NEAT) conference. AIDS 2011;25(4) 399-409

3.Wilkins, E. HIV Medicine (2013), 14 (Suppl. 4), 1–71. British HIV Association guidelines for the management of hepatitis viruses in adults infected with HIV 2013

4.Nastouli E. Journal of Clinical Virology 2009;44(1):78-80. Diagnosing acute hepatitis C in HIV-infected patients: nucleic acid testing compared with antibody and antigen-antibody detecting methods.

5.Medici M.C. Journal of Clinical Virology 2011;51(4):264-9. Medici M.C. Journal of Clinical Virology 2011;51(4):264-9.

6.Asboe et al. HIV Medicine (2012), 13, 1–44. British HIV Association guidelines for the routine investigation and monitoring of adult HIV-1-infected individuals 2011

7. Seme, K.S et al. J. Clin. Virol. (2005); 92-101. The role of core antigen detection in management of hepatitis C: a critical review
8. Chakravarti A. Brazilian J. Infect. Dis. 2013;17(3):369-74.

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