Journal of Clinical Virology 57 (2013) 29–35

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

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

A hepatitis C avidity test for determining recent and past infections in both plasma and dried blood spots

Samantha J. Shepherd[a,*], Joy Kean[a], Sharon J. Hutchinson[b,c], Sheila O. Cameron[a], David J. Goldberg[b], William F. Carman[a], Rory N. Gunson[a], Celia Aitken[a]

[a] West of Scotland Specialist Virology Centre, Gartnavel General Hospital, 1053 Great Western Road, Glasgow, United Kingdom
[b] Health Protection Scotland, NHS National Services Scotland, Meridian Court, 5 Cadogan Street, Glasgow, United Kingdom
[c] Department of Mathematics and Statistics, University of Strathclyde, Glasgow, United Kingdom

ARTICLE INFO

Article history:
Received 31 August 2012
Received in revised form
26 November 2012
Accepted 3 January 2013

Keywords:
Avidity
HCV
DBS
Recent
Chronic
Resolved

ABSTRACT

Background: DBS testing has been used successfully to detect HCV antibody positive individuals. Determining how long someone has been infected is important for surveillance initiatives. Antibody avidity is a method that can be used to calculate recency of infection.
Objectives: A HCV avidity assay was evaluated for both plasma and DBS.
Study design: To measure antibody avidity a commercial HCV ELISA was modified using 7 M urea. The plasma samples were split into: group 1 (recently infected $N = 19$), group 2 (chronic carrier $N = 300$) and group 3 (resolved infection $N = 82$). Mock DBS made from group 1 ($N = 12$), group 2 ($N = 50$), group 3 ($N = 25$) and two seroconverter panels were evaluated. 133 DBS taken from patients known to have a resolved infection or be a chronic carrier were also tested.
Results: The avidity assay cut-off was set at AI ≤ 30 for a recent infection. Using sequential samples the assay could detect a recent infection in the first 4–5 months from the point of infection. Most of the false positive results (AI < 30 among cases known not to have had recent infection) were detected among known resolved infections, in both the plasma and DBS; as a result, a testing algorithm has been designed incorporating both PCR and two dilution factors. The sensitivity and specificity of the assay on plasma was 100% and 99.3%, respectively, while DBS had 100% sensitivity and 98.3% specificity.
Conclusion: The HCV avidity assay can be used to distinguish between chronic and recent infection using either plasma or DBS as the sample type.

1. Background

In Scotland, approximately 1% of the population are hepatitis C virus (HCV) antibody (anti-HCV) positive and a significant proportion acquired infection through injecting drug use.[1] It can be difficult to obtain a conventional blood sample from people who inject drugs (PWID) and dried blood spot (DBS) testing is a non-invasive method that can be used for antibody and PCR testing.[2–4] In specialist drug service settings in Scotland, it has contributed greatly to the rise in the number of HCV diagnoses.[1] DBS testing has also been used successfully for surveillance purposes.[5–7]

Treatment of early HCV infection is associated with higher sustained virological response rates, including among PWID.[8–10] The ability to distinguish between a recently acquired and a past infection is important to help guide the clinical management of patients with HCV and direct prevention initiatives.[6]

A recently acquired HCV infection is often asymptomatic.[10] Traditionally a recent HCV infection is determined by monitoring antibody IgG seroconversion, and/or the detection of viral RNA in the absence of anti-HCV. It can take between 1–4 weeks for RNA and 8–12 months for antibody to become detected after infection with HCV.[11] The presence of anti-HCV and HCV PCR can indicate either an acute or a chronic infection, even in the presence of elevated liver function tests.[11] IgM antibody in HCV can be detected during exacerbation of chronic HCV.[12] An anti-HCV positive and PCR negative result usually indicates a resolved HCV infection, but can also indicate an acute infection with low viraemia.[11]

Antibody avidity is the binding capacity of maturing antibody with antigen, which increases over time. A dissociation agent (DA) can remove weakly bound antibody.[13] The avidity index (AI) can be measured using a modified enzyme linked immunoassay (ELISA) by comparing an untreated sample and one treated with the DA.[14] Antibodies generated early in infection have weak antigen-binding capacity compared to a matured antibody generated against the

* Corresponding author at: West of Scotland Specialist Virology Centre, Gartnavel General Hospital, 1053 Great Western Road, Glasgow G12 0YN, United Kingdom. Tel.: +44 141 2110080.
E-mail address: Samantha.Shepherd@ggc.scot.nhs.uk (S.J. Shepherd).

1386-6532/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jcv.2013.01.002

Table 1
Characteristics of the patient groups used in this study.

Patient group	Number of samples	Average age (range)	Average anti-HCV (range)	Average viral load IU/ml (range)	Number of patients with each genotype				
					1	2	3	4	NT
Group 1 (recent) plasma/serum	19	34(20–62)	9.54 (5.65–16.09)	2,436,152[a] (948–16,900,000)	7	3	5	0	4
Group 1 (recent) mock DBS	12	34(20–61)	10.83 (5.65–16.09)	132,671 (948–384,518)	3	3	3	0	3
Group 2 (chronic) plasma/serum	300	42(5–77)	12.93 (6.35–17.97)	1,178,851[b] (1280–9,760,000)	152	14	108	2	24
Group 2 (chronic) mock DBS	50	42(22–70)	12.51 (8.89–15.15)	1,007,687[c] (3686–8,330,000)	25	3	17	2	3
Group 3 (resolved) plasma/serum	82	39(23–60)	10.86 (3.17–17.14)	N/A					
Group 3 (resolved) mock DBS	25	42(25–60)	11.79 (6.13–14.92)	N/A					
Group 4 (chronic) patient DBS	65	39(23–74)	>3[d]	PCR qualitative only					65[e]
Group 5 (resolved) patient DBS	68	37(25–60)	>3 for 36 samples 1.78 (0.89–2.99) for 42 samples	N/A					

[a] 4 samples were tested by a qualitative PCR method, 2 samples tested by Abbott Architect HCV core assay and 2 patients had a viral load < 1000 IU/ml (limit of detection of the HCV PCR was 50 IU/ml).
[b] 33 chronic patients had a viral load < 1000 IU/ml (limit of detection of the HCV PCR was 50 IU/ml).
[c] 6 chronic patients had a viral load < 1000 IU/ml (limit of detection of the HCV PCR was 50 IU/ml).
[d] Anti-HCV assay for DBS is the Ortho manual ELISA (see study design). Any sample with an OD > 0.8 is considered positive. All samples had an OD of >3 with the exception of one sample (OD = 1.980).
[e] HCV genotype is not currently tested on DBS.

same antigen. Several papers have addressed HCV avidity testing but all have been described using plasma/serum samples.[15–19] All the HCV avidity methods reported are modifications of commercial or in house ELISA kits using either guanidine or urea as the DA. The method described in this paper uses a modified commercial assay with an overnight incubation stage for HCV avidity testing. This assay can be used for both plasma/serum and DBS. To the best of our knowledge HCV avidity has not been reported using DBS.

2. Objectives

The aim of this study was to evaluate a HCV avidity test on plasma/serum and DBS for use in a routine laboratory as both a diagnostic and epidemiological surveillance tool.

3. Study design

3.1. Plasma and serum samples

Plasma or serum samples sent to the West of Scotland Specialist Virology Centre (WoSSVC) for routine anti-HCV and HCV PCR testing which had been stored at −80 °C were used in this study. All plasma/serum samples were screened for anti-HCV by the Abbott Architect Anti-HCV assay. The samples to be tested were grouped into recent, chronic and resolved infections (Table 1). Group 1 consisted of 19 patients samples with a recent HCV infection, defined as having either an anti-HCV positive result within (a) 4–6 months of a previous anti-HCV negative result, or (b) within 1 month of a previous anti-HCV negative and PCR positive result. All of group 1 were HCV PCR positive. Two of the patients within group 1 were renal patients involved in a HCV outbreak. These 2 patients had 8 and 5 follow up samples taken sequentially over a 5 and 6 month period, respectively. Three other patients within group 1 had a follow up sample. Group 2 consisted of 300 patients with chronic HCV, defined as those who tested anti-HCV and HCV PCR positive for greater than 1 year. Group 3 consisted of 82 patients with resolved HCV infection, defined as those who had tested anti-HCV positive and HCV PCR negative for an average of 6 years (range 1–14 years). Information on the number of patients treated, the duration of HCV infection or if HCV reinfection occurred was unknown for both groups 2 and 3.

Two seroconvertor panels PHV901 ($N = 10$) and PHV917 ($N = 9$) were obtained from Alere™ (Cheshire, UK), the genotypes for the panels were 1a and 2b, respectively.

3.2. Mock DBS

HCV negative whole blood was centrifuged in 200 μl aliquots at 5000 rpm for 5 min, after which 60 μl of plasma was removed and replaced by 60 μl of known HCV positive plasma. The samples were re-suspended and 50 μl was then spotted onto Whatman 903 Protein Saver cards and allowed to dry at room temperature for over 1 h. Twelve of the samples from group 1, 50 samples from group 2, 25 samples from group 3 and seroconvertor panels PHV901 and PHV917 were made into mock DBS samples. The DBS were stored at 4 °C until use.

3.3. Patient DBS samples

DBS sent to the WoSSVC for anti-HCV and HCV PCR testing and subsequently stored at 4 °C were used in this study. The samples came from clinics run by community addition services. Group 4 consisted of 65 DBS taken from known chronic patients (tested anti-HCV and HCV PCR positive for >1 year) and group 5 consisted of 68 DBS from resolved patients (tested anti-HCV and HCV PCR negative for an average of 6 years, range 1–14). The patients chosen for each group had had previous HCV results from plasma samples on record at WoSSVC. Information on the number of patients treated and the duration of HCV infection was unknown for both groups 4 and 5.

3.4. HCV antibody testing of DBS

The ORTHO HCV 3.0 ELISA Test System with Enhanced SAVekit (Ortho Clinical Diagnostics) was used to detect anti-HCV in DBS