

False negative immunoblot result during long period before seroconversion in a hepatitis C virus infected intravenous drug user

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Introduction

Among HCV-infected individuals in the general population, the interval between the detection of HCV RNA and the development of HCV antibodies is usually 5 to 6 weeks. However in rare cases seroconversion may be prolonged by up to 6 to 9 months [1]. In some individuals who have weak or restricted virus-specific antibody responses, such as intravenous drug users, immunosuppressed patients and some cases of cryoglobulinaemia, antibody response may be delayed for more than 12 months and low levels of HCV RNA may be present in blood during prolonged antibody-undetectable periods before seroconversion [1–5].

In such patients, who are very frequently asymptomatic, the only sign of liver impairment encountered during a routine check-up is slightly elevated alanine aminotransferase (ALT) and HCV infection is often not systematically and properly excluded in such circumstances [6].

HCV infection is currently diagnosed by the presence of specific antibodies identified by the association of a reactive screening assay and confirmation by recombinant immunoblot assay (RIBA), with or without the presence of detectable viral antigen or RNA [7–9].

In the 2003 guidelines of the Centers for

Disease Control and Prevention (CDC) for laboratory testing and result reporting of antibody to hepatitis C virus an anti-HCV-negative result is defined as 1) anti-HCV screening-test-negative; or 2) anti-HCV screening-test-positive, recombinant immunoblot assay (RIBA)-negative; or 3) anti-HCV-screening test-positive, NAT negative, RIBA-negative. According to the CDC laboratory algorithm for antibody to hepatitis C virus (anti-HCV) testing and result reporting, screening test positives for anti-HCV with low signal-to-cut-off (S/CO) ratios are confirmed by RIBA and, if RIBA is negative, they are reported as negative and the screening test result is considered as false reactive or non-specific and such persons are considered uninfected [10]. Following this algorithm, false negative results in some patients, such as IDUs, are possible during the prolonged period before seroconversion.

Case report

In our case a 21-year-old female intravenous drug user (IDU) with a manic-depressive psychosis and a history of prostitution, had a routine check up in November 2003 in another practice and laboratory setting. At that time the only pathological result was a weak reactive HCV-EIA screening test, followed by a negative HCV immunoblot. According to the guidelines of the Centers for Disease Control and Prevention (CDC) for laboratory testing and result reporting of antibody to hepatitis C virus, the result was interpreted as anti-HCV-negative and the screening test result was interpreted as false reactive.

The patient presented to us in February 2004 for a follow up check up. A fourth-generation HIV-1/2 antigen/antibody EIA screening test gave a negative result.

Antibodies against hepatitis B surface antigen (anti-HBs) in the absence of hepatitis B core antibodies (anti-HBc) and antibodies against hepatitis A virus were detected and this result was consistent with the known im-

munization history. An HCV third-generation EIA screening test (HCV EIA Version 3.0 Axym, Abbott) gave a borderline reactive result but confirmatory immunoblot tests (DeciScan HCV Plus, Bio-Rad Laboratories and InnoLia HCV Score, Innogenetics) did not show any visible reaction and these results were comparable to, and showed no progression from, the previous testing in the other practice setting in November 2003.

Total bilirubin, alkaline phosphatase, gamma-GT (GGT) and aspartate aminotransferase (AST) were within the normal range and only slightly elevated alanine aminotransferase (ALT, 48 µmol/L, previous examination 31 µmol/L; elevated values >35) indicated a possible liver impairment. No vomiting, diarrhoea or fever were reported. The patient reported that, from time to time, she felt tenderness and slight pain in the upper right quadrant of her abdomen.

Keeping in mind that HCV antibodies usually become detectable only after a significant time delay following infection, a control testing was recommended. In a second sample, taken two months later, a significant increase in the HCV EIA S/CO ratio was observed but both immunoblots remained negative. According to the HCV CDC confirmatory algorithm, because of negative results from both immunoblot assays, an HCV infection was unlikely and again the screening test would be considered false reactive [10]. Due to the slightly elevated ALT and S/CO ratio increase in EIA we decided to perform a PCR test in both samples and found 14,600 IU/mL of HCV RNA already present in the first sample and 226,000 IU/mL in the second sample [11]. At this stage an active HCV infection was confirmed and another sample was requested to follow up on the immunoblot performance. A seroconversion in both immunoblots was encountered in a sample from October 2004 and an acute HCV infection with a significant time delay was also serologically confirmed. In a sample collected one year later slightly elevated ALT values were still noted, comparable EIA S/CO ratio and a quantitative HCV-PCR gave a result of 163,000 IU/mL. With this result a diagnosis of chronic HCV infection, which appears in around 80% of acutely infected persons, was made [2]. Genotyping showed an infection with genotype 3a, which is typically grouped in Switzerland in the IDU community [12]. The clinical picture over the monitoring period remained unchanged and laboratory results did not show any progression.

Discussion and conclusions

In our case an HCV third-generation EIA screening test gave a borderline reactive result but a confirmatory immunoblot tests did not show any visible reaction. In a second sample, taken two months later, a significant increase in the HCV EIA S/CO ratio was observed but both immunoblots remained negative. The difference in the test sensitivity

Table 1

False negative immunoblot results as a potential cause of missing a hepatitis C virus infection in an intravenous drug user.

Collection date	ALT Cobas Integra 800, Roche Diagnostics*	HCV EIA AxSYM, version 3.0, Abbott Diagnostics**	DeciScan HCV Plus Immunoblot, Bio-Rad Laboratories	INNO-LIA HCV Score, Innogenetics	HCV PCR Cobas Amplicor HCV Monitor Test, version 2.0, Roche Diagnostics*
February 13, 2004	48	1.3	negative	negative	14 600 IU/mL
April 29, 2004	76	11.54	negative	negative	226 000 IU/mL
October 6, 2004	95	66.40	positive	no serum available	323 000 IU/mL
October 4, 2005	51	66.50	positive	positive	163 000 IU/mL

* elevated >35 ** cut off 1.0

observed in this case between EIA and immunoblot could be explained by lower sensitivity of some immunoblot assays and could be an explanation for the "delayed seroconversion" [13]. An additional explanation for the difference in sensitivity between the EIA and the immunoblots observed in this study could possibly be related to HCV genotype 3a which is typically grouped in Switzerland in the IDU community and has to be further elucidated [12].

This case shows that false negative HCV immunoblot results after reactive screening results are possible. Following the CDC Laboratory algorithm for antibody to hepatitis C virus (anti-HCV) testing and result reporting, interpreting false negative immunoblot as true negative can lead to missing an active HCV infection in some individuals who have weak or restricted virus-specific antibody responses, such as intravenous drug users. Therefore we suggest that such persons cannot be considered uninfected solely on the basis of a negative immunoblot result. To avoid missing an active HCV infection in IDUs, a negative immunoblot result should be followed by nucleic acid amplification testing (NAT).

This approach would optimize early HCV diagnosis in terms of opportunities for

earlier treatment, source identification and introduction of preventive measures.

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