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Early Detection of Hepatitis C Virus Infection by Use of a New Combined Antigen-Antibody Detection Assay: Potential Use for High-Risk Individuals

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The performance of a new combined antigen-antibody assay (Monolisa HCV Ag-Ab Ultra; Bio-Rad Laboratories) was evaluated in the context of acute hepatitis C in human immunodeficiency virus-infected patients. The combined assay became positive as early as the first PCR and earlier than a third-generation enzyme-linked immunosorbent assay in 65% of the cases. Reduction of the long period of HCV seronegativity should improve the diagnosis of hepatitis C infection, especially in high-risk populations.

The early diagnosis of hepatitis C virus (HCV) infection is crucial to prevent further transmission in high-risk groups and to allow clinicians to make a rapid decision about treatment, which has been proven to have a high degree of efficacy for acute hepatitis C (5). Due to the long period of seronegativity, serological assays (18) remain poorly efficient for diagnosis in the early stages of HCV infection. Strategies that can be used to improve the ability to diagnose the infection before seroconversion require additional direct tests, such as detection of viral RNA by PCR or detection of HCV core antigen (Ag). However, genomic tests, which are limited by the requirement for specific equipment, a long delay in the time to a result, the risk of contamination, and high costs, cannot be used as routine screening tools. Moreover, the assay based on detection of the HCV core protein (Trak-C; Ortho Clinical Diagnostics) (12) and proposed as an alternative to PCR (3, 19) is no longer available in several countries and suffered from a lack of sensitivity and relatively long hands-on time. The simultaneous detection of both antigen and antibody (Ab) is now commonly used for the diagnosis of human immunodeficiency virus (HIV) infection, with the result being a dramatic shortening of the so-called seronegative window and, subsequently, the earlier diagnosis of recent infection. A similar approach based on the HCV core protein and specific anti-HCV antibody detection (Monolisa HCV Ag-Ab Ultra; Bio-Rad Laboratories, Marnes-la-Coquette, France) has recently been developed for the diagnosis of hepatitis C. The overall performance of this combined assay has previously been evaluated with seroconversion panels and HCV RNA-positive HCV Ab-negative samples (2, 10, 11).

In the context of HIV infection, the HCV seroconversion delay is often prolonged (16), and the failure of reactivity of current HCV Ab detection tests (9, 15) often requires PCR for HCV RNA detection. The aim of this study was to complete previous evaluations of the Monolisa HCV Ag-Ab Ultra assay (2, 10, 11) by assessing its potential clinical benefit in the setting of acute HCV infection in HIV-infected patients.

Twenty HIV-infected patients were recently identified to have acute hepatitis C. These patients were among the patients in our local cohort of HIV-infected homosexual males with high-risk sexual behaviors (6). The patients had been HIV infected for 5 to 19 years, all were receiving highly active antiretroviral therapy, and for most of them, the HIV infection was well controlled. Stored plasma samples were available from quarterly HIV viral load monitoring. Hepatitis C was first suspected by an increase in the alanine transaminase (ALT) level and/or the personal history of the patient and was further confirmed by two consecutive positive HCV RNA detections. HCV infection was retrospectively proven by the use of stored frozen serum and plasma samples: all cases were previously HCV seronegative and HCV RNA negative by PCR (COBAS AMPLICOR HCV Monitor test, version 2.0; Roche Diagnostics, Meylan, France) and later showed a positive result for HCV RNA, followed by seroconversion (detected by the Monolisa anti-HCV Plus V2 assay; Bio-Rad) for all but one of the patients. HCV genotypes were determined by sequencing of the NS5B region (17). The level of total HCV core antigen was measured by using the Trak-C immunoassay (Ortho Clinical Diagnostics). Samples were analyzed by the Monolisa HCV Ag-Ab Ultra assay (Bio-Rad), according to the manufacturer's recommendations. The results are expressed as the ratio of the absorbance to the cutoff; a ratio above 1 is considered a positive result. As suggested previously, we also considered a sensitized threshold (gray zone) at a ratio of 0.5 (2, 11).

We first confirmed with a cohort of 160 HIV-infected patients (80 were HCV seronegative and 80 were chronically coinfected with HCV and HIV) the overall excellent specificity and sensitivity of the Monolisa HCV Ag-Ab Ultra assay (data not shown). In this cohort, the proposed sensitized threshold ratio of 0.5 was above the mean value (0.011) for the seronegative samples plus 10 standard deviations.

Ninety-four samples from our panel of samples from 20 HIV-seropositive patients with acute HCV infections were tested by the combined Monolisa HCV Ag-Ab assay in parallel

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TABLE 1. HIV and HCV infection characteristics and performances of the Ag-Ab assay expressed as delays of HCV infection detection compared to those by reference methods

Patient no.	Age (yr)	HIV viral load (no. of copies/ml)[a]	CD4 count (no. of cells/mm^3)	First positive HCV PCR result ($\log_{10}$ IU/ml)	HCV genotype	Time (days) to result[b]				
						PCR, Ab	PCR, Ag-Ab		Ag-Ab, Ab	
							Threshold 1	Threshold 0.5	Threshold 1	Threshold 0.5
1	46	<200	668	6.75	4d	85	0	0	85	85
2	50	<200	670	4.65	3a	32	26	26	6	6
3	39	<200	681	6.08	1a	100	0	0	100	100
4	35	<200	389	>6.89	3a	37	9	0	28	37
5	50	39,000	410	5.97	4d	0	0	0	0	0
6	42	<200	618	5.64	2	0	0	0	0	0
7	55	<200	227	6.06	3a	5	0	0	5	5
8	47	<200	394	5.94	3a	78	0	0	78	78
9	53	<200	322	6.44	3a	0	0	0	0	0
10	51	<200	514	>6.89	1a	131	131	0	0	131
11	38	<50	657	6.67	4	–[c]	–	0	–	–
12	48	238	478	6.81	4d	62	0	0	62	62
13	53	<20	801	4.48	3	94	94	18	0	76
14	51	<200	214	>6.89	4d	106	0	0	106	106
15	31	<200	187	6.09	4d	148	99	0	49	148
16	34	<200	408	6.84	4	143	0	0	143	143
17	56	<200	582	>6.89	4d	89	0	0	89	89
18	61	1,960	355	<2.78	1a	126	126	126	0	0
19	46	75,900	439	6.71	1	56	0	0	56	56
20	31	2,570	660	6.49	4	93	0	0	93	93
Total	47[d]	200[d]	478[d]	6.58[d]		77[e]	27[e]	9[e]	50[e]	68[e]

[a] At presentation.
[b] PCR, first positive HCV RNA detection; Ab, first positive detection by Monolisa anti-HCV Plus V2; Ag-Ab, first positive detection by Monolisa HCV Ag-Ab Ultra.
[c] –, no seroconversion.
[d] Median.
[e] Mean.

with HCV RNA detection and anti-HCV Ab tests (Table 1). Detection by the Monolisa HCV Ag-Ab assay occurred earlier than by the Ab assay for 13 patients (65%). The mean delay of HCV infection detection by the new test was 27 days after the first positive result for HCV RNA detection (and 9 days when the optimized threshold of 0.5 was used), whereas it was 77 days by Ab detection alone. In our specific population, the Ab detection assay would then become positive on average 50 days after the Ag-Ab assay (and 68 days by use of the optimized threshold of 0.5). In 65% of the cases, the combined assay became positive with the same blood sample used for HCV RNA detection. With the optimized threshold of 0.5, reactivity at the time of HCV RNA detection was detected in 85% of the cases. Interestingly, for most patients (9 of 12) for whom follow-up ALT levels were available, the first detection of HCV infection by the combined assay occurred before the peak ALT level was detected (data not shown).

For patient 11, who had a prolonged seronegative period over 18 months, a lack of reactivity by the Ag-Ab assay was observed, despite high HCV viral loads (over 1 million IU/ml) in several consecutive samples. It is noteworthy that values between 0.5 and 1 were obtained for most samples. The core coding gene sequence was determined in two samples from this patient by direct sequencing: the core protein was 98% identical to the GI:532369 GenBank sequence, and no major change in the sequence from those of other genotype 4 core proteins was noticed (4). The core protein level in several samples was measured by the Ortho Trak-C assay and ranged from 39 to 406 pg/ml, which are values in accordance with the viral load levels (3). The production of antibody against each HCV protein was analyzed by an immunoblot assay, which eventually became positive.

Diagnosis of acute hepatitis C largely relies on classical serological methods (1). However, the value of such Ab detection assays is limited during the early stages of HCV infection, first, because specific antibodies develop slowly and, second, because immunocompromised patients may fail to develop a strong and rapid specific immune response against HCV. The development of new assays that combine both Ag and Ab detection, similar to what has been done in the field of HIV detection (13), may prove useful in reducing the long window of HCV seronegativity or compensating for the absence of a specific antibody response. This is particularly true in the case of HIV coinfection, where long periods of seroconversion have been described (14, 16). Those combined tests present a potential advantage and may improve commonly used serological assays without the need to resort to an additional direct detection procedure, such as PCR or Ag measurement.

In this evaluation of the Monolisa HCV Ag-Ab Ultra assay with samples from 20 HIV-infected patients who developed acute HCV infection, the new combined test allowed, in most cases, the detection of hepatitis C infection earlier than that by conventional serological assays. Obviously, calculation of delays between the reactivity of the serological assay and that of the combined assay would be biased in this study due to heterogeneous intervals between the times of testing of two consecutive samples from a patient and the long HCV-seronegative period usually observed in HIV-infected patients. Nevertheless, these long intervals are

those observed in the "real" clinical setting and in the management of HIV-infected patients. Of note, our detection delays are consistent with those previously calculated with either commercial (30 days) or so-called natural (28 days) seroconversion panels (2, 10). Importantly, for a majority of patients, the combined assay became positive as early as HCV RNA detection. In our population, the combined assay never failed at identifying a seroconversion that would have been detected by the Monolisa anti-HCV Plus assay.

Unexpectedly, for one patient who showed persistent HCV replication but an absence of seroconversion for more than 1 year, the combined assay led to only a weak response. Sequencing of the core encoding gene and quantification of the core protein by the Trak-C assay did not support any peculiarity of the HCV core protein, and reactivity by immunoblot testing was evidenced for this patient. Thus, the weak reactivity would reflect in this case a lack of sensitivity of the Monolisa HCV Ag-Ab Ultra assay for the detection of the core protein and anti-HCV Ab. Other hypotheses should be investigated, however, like presence of immune complexes or the interaction of the virus with lipid components. The low reactivity observed for this patient also reinforces the usefulness of establishing a gray zone ratio of 0.5 to 1 that may permit the better identification of samples in the seroconversion window, as previously suggested in two studies (2, 11). The sensitivity of Ag detection by the combined test might be less satisfying compared to that by HCV core Ag detection assays. However, the purpose of this new combined test is mainly to improve and eventually replace the serological assays used as first-line screening tools, whereas core Ag detection assays would rather be used as a replacement for nucleic acid testing (7, 8).

In conclusion, although the influence of the HIV infection status on HCV detection by the Monolisa HCV Ag-Ab Ultra assay needs to be confirmed with larger panels of samples, including samples from very immunocompromised patients, the results of this first study with samples from HIV-infected patients are promising. Similar to what has been demonstrated for the Ag-Ab combined assays used for the diagnosis of HIV infection, the use of such a combined assay for HCV detection brings a real clinical benefit compared to conventional serological assays by shortening the seronegative window period. Such an improvement may be particularly useful for the diagnosis of HCV infection in high-risk groups, where early infection detection may help reduce secondary transmissions and rapid referral for treatment. Because of its relatively low cost, its ease of performance, and the significant decreased window period, we believe that this new assay will improve the overall quality of diagnosis of HCV infection for a moderate overall cost compared to those of serological assays and should therefore be applied as a screening assay in the general population.

We must emphasize that this assay is not intended to replace HCV RNA detection, and in the case of acute clinical hepatitis, the use of a sensitive HCV RNA detection assay is highly recommended. Because the sensitivity of the HCV core detection assay is significantly lower than that of any PCR-based method, it should not be recommended for use for this particular purpose.

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