Serologic survey for control of hepatitis C in haemodialysis patients: third-generation assays and analysis of costs


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Abstract There is little information about the serologic survey for control of hepatitis C by using third-generation assays among chronic haemodialysis (HD) patients, and no analysis of costs has been made to this end. A serologic survey for control of hepatitis C was performed in 190 HD patients attending a single dialysis unit, using second- and third-generation assays. Costs of both serologic surveys were calculated. Anti-HCV prevalence tested by third-generation assays increased from 25% (48/190) to 28% (53/190) compared to second-generation testing; 56% (9/16) of patients showing uncertain findings by second-generation tests gave unequivocal results by third-generation assays; median duration of HD treatment and raised aminotransferase levels were positively associated (P=0.004 and P=0.012, respectively) with anti-HCV detected by third-generation assays. The serologic survey for control of hepatitis C in HD patients at our centre was slightly more expensive by third-generation assays compared to second-generation testing (US$18,866 vs US$17,200 per year). In summary, the use of third-generation tests largely clarified the uncertain results of second-generation tests; new additional positive patients were detected by third-generation assays compared to second-generation testing. Third-generation assays showed the association of duration of HD treatment and raised aminotransferase levels with anti-HCV antibody, as previously found by first- and second-generation assays. To date, third-generation screening and confirmatory assays seem extremely useful in the serologic survey for control of hepatitis C in HD centres without a considerable outlay.

Key words: third-generation HCV screening assays; third-generation HCV confirmatory assays; haemodialysis patients; analysis of costs

Introduction

Haemodialysis (HD) patients are a high-risk group for hepatitis C virus (HCV) infection. The prevalence of anti-HCV antibody ranged between 10% and 55% in reported series [1–18]. The Centers for Disease Control and Prevention (CDC) recently issued guidelines to control hepatitis C in HD centers [19]. They recommended that all patients should be monitored monthly for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels to detect any type of non-A, non-B (NANB) hepatitis. Elevations in liver enzymes are more sensitive indicators of acute infection than is detection of anti-HCV antibody. They also suggested conducting serologic screening for anti-HCV among HD patients in order to assess prevalence of HCV and determine medical management in patients with hepatitis C. The frequency of anti-HCV antibody has been determined by using first- and second-generation assays, but third-generation screening ELISA (Ortho HCV ELISA 3 test system) and immunoblot assay [RIBA® HCV 3.0 Strip Immunoblot Assay (SIA), Chiron Corporation] were introduced in June 1992. They were designed to improve both sensitivity and specificity of the assays by enlarging the array of HCV-encoded antigens included, thus favouring specific over non-specific antibody detection [20,21]. In fact, one of the manufacturers (Ortho) added an NS5 recombinant protein in both screening and confirmatory third-generation assays. Moreover, third-generation confirmatory assay (RIBA® HCV 3.0 SIA) uses two synthetic peptides instead of recombinant proteins such as in RIBA® HCV 2.0 SIA and the coating concentrations of RIBA® HCV 3.0 SIA proteins have been optimized compared to the previous RIBA® HCV 2.0 SIA.

Several authors [22,23] recently used third-generation tests for detecting anti-HCV antibody in HD patients, in small patient groups. We also need to assess the costs of serologic surveys for control of hepatitis C in HD centres. In an era of cost containment, we need to arrive at economic solutions that do not compromise the quality of care.
The aim of this study was to evaluate the epidemiology of anti-HCV antibody and assess the costs of serologic surveys for control of hepatitis C in HD patients at our centre by second- and third-generation tests.

Subjects and methods

Enzyme immunoassays

The ELISA-2 Ortho HCV ELISA test system detects antibodies to structural (core antigen) and a fusion of the c100–3 and c33c antigens (c200) of HCV. It uses recombinant antigens originating from three regions of the viral genome (core, NS3, and NS4). The ELISA-3 Ortho HCV ELISA test system uses three recombinant antigens (c22-3, c200, and NS5) originating from four regions of the viral genome (core, NS3, NS4 and NS5). All tests were carried out and interpreted strictly in accordance with the manufacturers’ instructions.

Confirmatory assays

RIBA® HCV 2.0 SIA is a recombinant immunoblot assay with four antigens (c22-3, c33c, c100-3, and 5–1–1) covering three regions of the viral genome (core, NS3, and NS4). RIBA® HCV 3.0 SIA is an immunoblot assay using two recombinant proteins (c33c, NS5) and two synthetic peptide (c22p, c100p) antigens covering four regions of the viral genome (core, NS3, NS4, and NS5). Results by RIBA® HCV 2.0 and 3.0 SIAs were interpreted according to other authors, as previously described [24].

RT-PCR

We used a simple and rapid PCR system (Amplicor HCV PCR kit, Roche Molecular Systems, Basel, Switzerland) based on combined transcription–amplification reaction and non-isotopic hybridization, as previously described [25].

Patients

We studied 190 HD patients, 111 males and 79 females, attending a single dialysis unit in Lecco, northern Italy in May 1994. The patients had been treated with chronic HD for end-stage renal failure for a median of 43 months (range 1–256); the mean age was 62.6 ± 13.3 years (range 20–89). Routine HD techniques were performed with 3- to 4-h treatments three times a week. Bicarbonate dialysis was used for all patients. The most common causes of renal failure were glomerulonephritis (in 59), nephrosclerosis (36), interstitial nephritis (22), diabetic nephropathy (22), and polycystic kidney disease (27). No patient admitted a history of intravenous drug abuse. Hepatitis B surface (HBsAg), hepatitis B e antigen (HBeAg), antibodies to hepatitis B surface (H BsAb), e (H BeAb), and core (HBcAb) antigens, and antibodies to the immunodeficiency virus were measured by commercially available kits from Abbott Diagnostics. No patient had detectable anti-human immunodeficiency virus in our unit in spite of the fact that some of them had received several blood transfusions. The history of blood transfusion requirement for each patient was evaluated.

Serum aminotransferase activities

Serum AST and ALT activities, determined by spectrophotometry every 3 months, were retrospectively evaluated after the onset of HD. The upper normal limits of AST and ALT concentrations are 46 and 40 U/l, respectively. Elevated levels were defined as greater than twice the value of the upper limit of the reference range. The epidemiological features of HCV infection by first- and second-generation assays in our HD patients were previously studied [6,10].

Study protocol

All the patients were tested by second- and third-generation screening assays (ELISA-2 and ELISA-3). All ELISA-2-positive patients were tested by second-generation confirmatory assay (RIBA® HCV 2.0 SIA). The cohort of patients showing equivocal findings with second-generation assays, i.e. ELISA-2-positive and RIBA® HCV 2.0 SIA-indeterminate patients (presence of one HCV band alone), was evaluated with third-generation confirmatory assay (RIBA® HCV 3.0 SIA) and RT-PCR. From the group of patients showing unequivocal findings with second-generation assays, i.e. ELISA-2-positive RIBA® HCV 2.0 SIA-positive patients (presence of at least two HCV bands), a subgroup of patients was selected at random and evaluated by third-generation confirmatory assay (RIBA® HCV 3.0 SIA). Additional positive sera by ELISA-3 were analysed by second- and third-generation confirmatory tests (RIBA® HCV 2.0 and 3.0 SIA) and by RT-PCR.

Analysis of costs

We calculated the costs of the serologic survey for control of hepatitis C in chronic HD patients at our centre per year. The costs of serologic tests were based on the prices paid by the medical centre for the test kits. These prices reflect the actual cost of the products to the hospital. No labour cost was included in our analysis, since dialysis patients receive intense physician care and undergo regular diagnostic tests; the additional labour cost associated with serologic testing is negligible. Our analysis did not regard staff members at our unit. We have made several assumptions to facilitate the analysis: (1) a US$3 charge for second-generation anti-HCV screening test and a US$50 charge for second-generation anti-HCV confirmatory assay; (2) a US$5 charge for third-generation anti-HCV screening test and a US$62 charge for third-generation anti-HCV confirmatory assay; (3) a US$3 charge for serum ALT testing and a US$3 charge for serum AST testing; (4) a US$69 charge for detection of serum HCV RNA (Amplicor HCV test, Roche Molecular Systems, Basel, Switzerland) and a US$81 charge for HCV genotyping by a hybridization assay (LiPA 2, Zwichendrecht, Belgium); (5) all chronic HD patients at our centre (190 patients) are tested semiannually for anti-HCV screening assay, whereas anti-HCV positive patients are confirmed by anti-HCV confirmatory assays once a year; (6) the anti-HCV prevalence is 25% (48/190) by second-generation assays and 28% (53/190) by third-generation assays; (7) the prevalence of detectable HCV RNA in serum of anti-HCV positive patients is 44% (22/50) [26].

Statistical methods

Results are expressed as means ± SD. Group comparisons were made by chi-square test with Yates’ correction or
Fisher’s exact test (for dichotomous variables) and a Mann–Whitney test for non-parametric values. A P value less than 0.05 was accepted as significant. Statistical analysis was performed with the computer program package Primer (by Stanton A. Glantz, 1989).

Results

Third-generation screening assays

The prevalence of anti-HCV antibodies was 25% (48/190), using the second-generation screening assay. All ELISA-2-positive patients showed reactivity by RIBA HCV 2.0 SIA: the results are shown in Table 1. All ELISA-2-positive patients gave positive results with the ELISA-3 screening test. Among the ELISA-2-negative patients, there were 18 ELISA-2-negative sera which gave positive results by ELISA-3. A follow-up sample from each patient obtained 1 month later gave identical results in ELISA-2 and ELISA-3 screening assays.

Third-generation confirmatory assays

The additional positive patients by ELISA-3 were evaluated by RIBA® HCV 2.0 and 3.0 SIA. RIBA® HCV 2.0 SIA showed one positive, four indeterminate and 13 negative samples. RIBA® HCV 3.0 SIA gave four positive, two indeterminate and 12 negative samples. Thus, among the additional positive sera by ELISA-3, six were considered truly positive and 12 were considered false positive results as they did not show reactivity with both the recombinant immunoblot assays (RIBA® HCV 2.0 and 3.0 SIA). Table 2 shows the pattern of reactivity against specific HCV proteins using RIBA® HCV 2.0 and 3.0 SIA in new additional positive sera by ELISA-3. One patient reacted with NS5 band of RIBA® HCV 3.0 SIA; the other patients reacted with c22p and/or c33c lanes of RIBA® HCV 3.0 SIA. No patient reacted with c100p lane of RIBA® HCV 3.0 SIA.

Four of the six additional positive patients by ELISA-3 had previously given conflicting results: they alternated between very weakly positive and negative results by ELISA-2 screening test. They were definitively positive in third-generation assays.

We evaluated by RIBA® HCV 3.0 SIA the subset of 16 patients giving unclear findings with second-generation assays (Table 3): eight (50%) out of 16 patients showed positivity (reactivity to at least two bands on RIBA® HCV 3.0 SIA); seven (44%) out of 16 showed reactivity against one lane of RIBA® HCV 3.0 SIA. Table 4 shows the pattern of reactivity against specific HCV proteins using RIBA® HCV 2.0 and RIBA® HCV 3.0 SIA in a such cohort of patients. One patient showing a low reactivity (1+ with c22-3 lane by RIBA® HCV 2.0 SIA tested negative by RIBA® HCV 3.0 SIA. He was ELISA-3 positive and RIBA® HCV 3.0 SIA negative and was considered false positive. Thus, nine (56%) out of 16 patients with unclear results by second-generation findings showed unequivocal results with third-generation assay.

Prevalence of anti-HCV-positive patients increased to 28% (53/190) using third-generation tests in our HD population. We tested with RIBA® HCV 3.0 SIA a cohort of 15 patients giving clear positivity by second-generation tests: seven out of 15 reacted with NS5 lane; an additional c100p band was observed in six sera. All patients showed a good reactivity against c22-3 (or c22p) and c33c lanes with both RIBA® HCV 2.0 and 3.0 SIA (15 × c22-3 and 15 × c22p, 15 × c33c).

Table 1. Results of second-generation confirmatory assay (RIBATM HCV 2.0 SIA) in 48 patients reactive by second-generation screening assay

<table>
<thead>
<tr>
<th>RIBATM HCV 2.0 SIA band pattern</th>
<th>No. (%) of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four band (positive)</td>
<td>10 (21)</td>
</tr>
<tr>
<td>Three band (positive)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Two band (positive)</td>
<td>18 (38)</td>
</tr>
<tr>
<td>One band (indeterminate)</td>
<td>16 (33)</td>
</tr>
<tr>
<td>No band (negative)</td>
<td>0</td>
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</tbody>
</table>

Table 2. Comparison between RIBATM HCV 2.0 and 3.0 SIA for HCV infection in new additional positive patients by ELISA-3

<table>
<thead>
<tr>
<th>No.</th>
<th>ELISA-2</th>
<th>ELISA-3</th>
<th>RIBATM HCV 2.0 SIA</th>
<th>RIBATM HCV 3.0 SIA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>c33c</td>
<td>c22-3</td>
<td>c100-3</td>
<td>5-1-1</td>
</tr>
<tr>
<td>1</td>
<td>Neg</td>
<td>Pos</td>
<td>1+</td>
<td>–</td>
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<td>6</td>
<td>Neg</td>
<td>Pos</td>
<td>1+</td>
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Table 3. Results of third-generation confirmatory assay (RIBATM HCV 3.0 SIA) in 16 ELISA-2-positive and RIBATM HCV 2.0 SIA-indeterminate patients

<table>
<thead>
<tr>
<th>RIBATM HCV 3.0 SIA band pattern</th>
<th>No. (%) of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four band (positive)</td>
<td>3 (19)</td>
</tr>
<tr>
<td>Three band (positive)</td>
<td>3 (19)</td>
</tr>
<tr>
<td>Two band (positive)</td>
<td>2 (12)</td>
</tr>
<tr>
<td>One band (indeterminate)</td>
<td>7 (44)</td>
</tr>
<tr>
<td>No band (negative)</td>
<td>1 (6)</td>
</tr>
</tbody>
</table>
Epidemiology of HCV infection by third-generation assays

The presence of anti-HCV as determined by ELISA-3 and RIBA® HCV 3.0 SIA was not associated with age, gender, serological positivity for HBV markers and transfusional requirement. There was a significant difference between anti-HCV-positive and -negative patients with regard to median duration of HD treatment and frequency of raised aminotransferase levels (Table 5).

Comparison of serum aminotransferase levels and anti-HCV antibody

Among 190 HD patients, 25 (13%) showed raised AST and ALT concentrations; 16 of them (64%) exhibited multiple peaks. They did not show other reasons for abnormal liver tests. However, at the time of the study, all but two patients had AST and ALT levels in the normal range. Thirteen out of 25 (52%) were anti-HCV positive; the frequency (Table 5) of raised aminotransferase levels was significantly higher in anti-HCV-positive than in anti-HCV-negative patients. Among 13 anti-HCV-positive patients with raised aminotransferase levels, nine were positive by second-generation tests, and four gave unclear results (ELISA-2 positive and RIBA® HCV 2.0 SIA indeterminate). Among the patients with unclear results by second-generation tests and raised aminotransferase values, two individuals gave positivity by ELISA-3 and RIBA® HCV 3.0 SIA.

HCV RNA detection by RT-PCR

Fourteen out of 16 patients with indeterminate results by second-generation tests were tested by RT-PCR; four showed detectable HCV RNA in serum, two of them being RIBA® HCV 3.0 SIA positive (reactivity against c22p and c33c) and two being RIBA® HCV 3.0 SIA indeterminate. The new additional positive sera by ELISA-3 showed normal aminotransferase values and no detectable HCV RNA in serum.

Analysis of costs

As shown in Table 6, the cost of the monthly determination of serum AST and ALT levels is US$13 680 in 190 patients at our centre per year. The total cost of the serologic survey was US$18 866 and US$17 220 respectively in our HD patients per year; the use of third-generation assays produced an increase of costs of US$1646 per year. The cost of the serologic survey (by third-generation assays) for control of hepatitis C for each patient per year is US$99. The cost of HCV RNA determination by RT-PCR and HCV genotyping is US$4546 in 190 HD patients per year. The total cost of the serologic survey (by third-generation assays) for control of hepatitis C including RT-PCR analysis and HCV genotyping is US$23 412 in 190 HD patients per year.

Discussion

Using third-generation assays resulted in the detection of more ELISA anti-HCV-positive patients on chronic haemodialysis patients in our unit by second- and third-generation assays.
HD treatment at our unit. Some of them showed reactivity by immunoblot assays; thus, we observed an increase of prevalence of anti-HCV antibody from 25% to 28% in our HD patients. Other third-generation positive patients were not confirmed by immunoblot assays and did not show detectable HCV RNA in serum: they are probably false positive results. Therefore, it is possible that the potential gain in sensitivity by third-generation assays will be at the expense of a new variety of false positive results.

All new additional positive sera by third-generation tests were HCV RNA negative, and 71% of patients showing indeterminate findings with third-generation assays tested negative by RT-PCR. The clinical significance of these cases remains to be established. The third-generation ELISA-positive samples reactive by third-generation RIBA and negative by RT-PCR could have recovered from HCV infection or could have had fluctuating levels of viraemia or very low viraemic levels below the limits of detection of the RT-PCR. Moreover, a cause of HCV RNA negativity by RT-PCR in HCV-infected patients could be related to an exclusive localization of HCV in the liver or in other non-blood compartments. Alternatively, the choice of oligonucleotide primers is critical for a clinically useful RT-PCR procedure because of the extreme variability of the HCV genome [27]. On the other hand, results by RT-PCR should be interpreted with care as the reliability of RT-PCR for diagnosis of HCV viraemia remains a problem in several laboratories, as previously suggested [28].

The analysis of the results by RIBA® HCV 3.0 SIA showed that the antibody response to NS5 region-encoded antigens was not as prevalent as to other antigens. The improvement we made with RIBA® HCV 3.0 SIA over RIBA® HCV 2.0 SIA was largely obtained by an improved detectability of anti-c33c antibodies and not by the addition of the NS5 lane, as suggested by other authors [29]. The importance of the additional NS5 component in RIBA® HCV 3.0 SIA is unclear [29,30]. We also detected an increased reactivity against c100p antibody, although to a lesser degree. NS5 lane was described in several samples, but it had minor repercussions in sample allocation.

The cohort of patients with unclear results by second-generation assays (i.e. patients with indeterminate results by RIBA® HCV 2.0 SIA) showed to a great extent unequivocal findings with third-generation assays. There was one patient showing unclear results by second-generation assays (isolate and weak c22-3 reactivity) who was allocated as negative by RIBA® HCV 3.0 SIA: such a finding is in accordance with an our previous observation [31]. The number of patients with equivocal findings by second-generation assays is large in a high-risk population such as HD patients, and the significance of the ‘indeterminate’ seroreactivity is still uncertain [20,21,29]. Two ELISA-2-positive patients having unclear findings by RIBA® HCV 2.0 SIA exhibited positivity by RIBA® HCV 3.0 SIA and raised aminotransferase levels; thus, third-generation recombinant immunoblot assay accounted for elevated AST and ALT levels in such patients. On the other hand, two patients with uncertain results by RIBA® HCV 2.0 and 3.0 SIA showed raised aminotransferase levels and HCV RNA positivity: further studies are warranted to evaluate the significance of indeterminate results by third-generation recombinant immunoblot assays. It is possible, indeed, that some RIBA® HCV 3.0 SIA-indeterminate patients turn out to be positive with further assays.

The evaluation of risk factors for anti-HCV antibodies tested by third-generation assays among these HD patients confirmed our previous results obtained using first- and second-generation assays.

The cost of the serologic survey for control of hepatitis C in HD patients at our unit is slightly higher by third-generation than second-generation assays. Such a difference is very low compared to the total cost of the serologic survey for control of hepatitis C at our unit. On the other hand, two-thirds of the cost of the serologic survey for control of hepatitis C is attributable to the cost of monthly determination of AST and ALT levels, as recommended by the CDC. In the routine serologic survey at our centre we did not include labour costs associated with RT-PCR analysis and HCV genotyping. These procedures are not available in most clinical laboratories due to laborious performance characteristics and are not recommended by the CDC for purposes of infection control. On the contrary, we included the screening of HD patients at our unit for anti-HCV antibody in our serologic survey for control of hepatitis C. Such a procedure is only suggested by the CDC, but it is very important in order to assess HCV incidence in dialysis centres and to identify problems with infection control practices. The cost of the serologic survey for control of hepatitis C among HD patients at our centre, as recommended by the CDC, may be considered high; however, the cost of the serologic survey for each patient is lower than the cost of a 2-day hospitalization for liver biopsy at our hospital. Moreover, it is very low compared to the cost of screening and segregating HBV surface antigen-positive patients. These procedures have been previously recommended by the CDC in order to prevent HBV transmission in HD centres [32].

In conclusion, the serologic survey for control of hepatitis C by third-generation screening and confirmatory tests largely clarified the unclear results by second-generation tests; we detected new additional positive patients with third-generation screening and confirmatory assays; third-generation assays confirmed the association of duration of HD treatment and raised aminotransferase values with anti-HCV antibody, as previously found by first- and second-generation assays; the serologic survey by third-generation tests was slightly more expensive compared to the second-generation assays.

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Nephrology Department (Dir. Prof. C. Ponticelli) of the Hospital ‘Maggiore-Policlinico’, IRCCS, Milano, Italy. Dr Fabrizi is an assistant at the Nephrology Department of the Hospital of Lecco, Italy. At present he is a Research Fellow of the Society of Italian–American Nephrologists.

References