Diagnosis of hepatitis C in hemodialysis patients with end-stage renal disease (ESRD): what is the best strategy?
and those with ESRD, respectively. However, viremia can be detected during this period by molecular biology methods.\textsuperscript{10,11,12}

Currently, in the absence of elevations in ALT, anti-HCV testing is conducted every six months in dialysis units, and this periodicity is recommended to monitor the development of new HCV infections, with detection of acute infection in up to 97\% of those patients.\textsuperscript{13,14} Some studies have reported that 0.8 to 2.3\% of patients with ESRD have false-negative serology despite confirmation of viremia by molecular biology methods.\textsuperscript{15,16,17,19,20,21}

Molecular biology testing allows HCV identification in immunosuppressed individuals, such as patients with ESRD on HD, in up to three weeks. It has also been observed that qualitative polymerase chain reaction (PCR) can be negative in patients with ESRD on HD due to the viral load oscillation attributed to dialysis. The serum level of HCV ribonucleic acid (RNA) can be significantly reduced after HD. Therefore, blood samples for this test should be drawn before a dialysis session.\textsuperscript{22,23,24} However, in recent studies, low viremia in those patients was attributed to compromised immune response rather than to dialysis.\textsuperscript{25} Currently, two techniques for the detection of viral RNA, PCR and transcription-mediated amplification (TMA), with detection of 50 IU/mL and 10 IU/mL, respectively, and specificity close to 100\%, are available.\textsuperscript{21}

Some studies suggest potential benefits of the routine use of PCR in dialysis units, such as the identification of patients with positive viremia and false-negative serology, and the early detection of acute hepatitis C in cases of increased ALT.\textsuperscript{16,17,20,22} However, due to elevated costs, molecular biology tests should not be considered routine in dialysis units.\textsuperscript{10}

In patients with ESRD, the immune response is compromised. This has been attributed to uremia, association of multiple chronic disorders, and factors related with RRT, such as the modality (HD) and time on dialysis. End-stage renal disease and associated comorbidities cause anemia and malnutrition, which contribute to decrease the immune response, and HD can worsen those complications if proposed guidelines are not followed. Uremic patients produce low titers of neutralizing antibodies against surface antigens, increasing the risk of infection with hepatitis B and C virus, which would explain the presence of seronegative tests.\textsuperscript{26,27}

In the last decade, some studies analyzed diagnostic methods for hepatitis C in dialysis units. However, the best investigational approach for diagnosis of HCV infection in ESRD patients remains undetermined. The reliability of biochemical, serologic, and molecular biology tests is controversial. Thus, studies aiming at identifying the reliability of diagnostic methods for hepatitis C in this group of patients are justified. The main advantages of the early diagnosis of hepatitis C viral infection include institution of treatment in the acute stage of the disease, increase of the chances of therapeutic response, and the early identification of newly infected patients, allowing adoption of measures to control the infection dissemination in dialysis units. Even though the prevalence of hepatitis C is decreasing, the disease still has a great impact in this population, due to the risk of chronic evolution, and of exclusion of those patients from transplant lists, since this would impair profoundly their evolution and graft survival, and would not allow the appropriate treatment of hepatitis.

In the present study, we attempted to determine the accuracy of diagnostic methods to detect hepatitis C in patients with ESRD on HD with negative past serology for HCV; evaluate the reliability of anti-HCV serology (3rd generation EIA) in the early detection of HCV infection, in patients with ESRD on HD, compared to ALT and PCR; and evaluate the proper periodicity for anti-HCV testing in the early diagnosis of acute hepatitis C in those patients.

**Methods**

**Study design**

This is a descriptive study that uses the panel study method, with cross-sectional analyses during the follow-up period, undertaken in the city of Belo Horizonte, MG.

**Sample size determination**

To determine the sample size, the previously reported prevalence of hepatitis C in patients with ESRD on HD, which varies from 10 to 50\%, was considered; in Belo Horizonte a prevalence of 12\% was observed.\textsuperscript{5,8,28,29} The reported prevalence of false-negative serology (3rd generation EIA) for HCV varies from 0.8\% to 2.3\%;\textsuperscript{11,15,17} we used as reference a minimal prevalence of positive serology of 10\% for HCV in patients with ESRD on HD, standard deviation between 2 and 3\%; a sample size of 500 (43.9\%) was representative, i.e., it corresponded to 24.23\% of the population on HD in Belo Horizonte, from a total of 2,063 patients distributed in 13 dialysis units in the city. Six dialysis units were chosen, through simple random drawing, to participate in the study, with a total of 1,266 patients, of which 1,138 (89.8\%) had negative anti-HCV and were eligible for the study; after further drawing, 500 patients, distributed in six dialysis units, were selected. Figure 1 shows the breakdown of the study population.
Inclusion and Exclusion Criteria

Inclusion criteria were as follows: patients on HD for more than three months, with arteriovenous fistula, of any age group, race, and gender, and any level of schooling; negative serology for HCV, HBV, and HIV in the three months before the study, at the moment of the drawing, and in the three consecutive months; availability of monthly ALT levels, and anti-HCV at the beginning of the study (month -3), at the time of collecting blood sample for molecular biology testing (month zero), and at the end of the six-month follow-up period (month +3).

Patients with a prior diagnosis of acute viral hepatitis or acquired immunodeficiency syndrome; patients on PD; venous access with double-lumen catheter; hospitalized patients with severe illness; patients discharged from HD after a kidney transplant; or those who died after the beginning of the study, were excluded.

Follow-up Protocol

Patients with three negative monthly serologies (month -3, month -2, and month -1) for HCV (anti-HCV, ELISA, 3rd generation) were included in the study. Blood samples were collected for molecular biology testing (qualitative HCV RNA PCR, Amplicor HCV Roche 2.0) on month zero, on the demarcation of the anti-HCV immunological window for three months (month +1, month +2, and month +3), and monthly ALT (Bioclin), which is shown in Figure 2. Table 1 shows the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each test.

Patients were followed for a three-month period before and after data and blood sample collection for molecular biology testing to detect HCV. This follow-up period represents the time necessary for seroconversion in the acute phase of HCV infection, including the im-

Figure 1. Study population.

2,063 dialysis patients distributed in 13 Dialysis Units in Belo Horizonte

Drawing of 6 dialysis units (DU)

DU1, DU2, DU3, DU4, DU5, DU6 1,226 patients

128 Anti-HCV+ patients excluded

1,138 anti-HCV-

DU1 288
DU2 200
DU1 163
DU1 153
DU1 114
DU1 220

Drawing of 500 patients

DU1 122
DU2 89
DU1 101
DU1 74
DU1 63
DU1 81

45 excluded

n = 455 patients

Figure 2. Patient follow-up.
munological window, since anti-HCV becomes positive seven to 12 weeks after infection in patients with ESRD on dialysis.23 Epidemiological data were obtained through questionnaires and patient records available in the dialysis units.

**BLOOD COLLECTION PROTOCOL**

Blood samples were collected passively, without vacuum, through venous access catheters immediately after the connection of the arterial venous fistula and before the administration of heparin. Blood was collected in 4.5 mL EDTA (ethylene diamine tetraacetic acid) containing tubes that were stored in thermal storage boxes containing gel ice packs and sent immediately to the molecular biology laboratory for processing and storage. Preparation of all blood samples was performed within two hours after collection. Blood samples were centrifuged at 4,000 rpm for 10 minutes in a Herme Z 323 centrifuge; blood aliquots were identified, divided in two one-milliliter fractions, and stored at –70°C until molecular biology testing.

**STATISTICAL ANALYSIS**

Sample size calculation was based on prevalence studies described above. Descriptive analysis was undertaken using central tendency (mean) and variability (standard deviation) measurements that demonstrated the characteristics of the findings, and only the anti-HCV NPV was calculated, since high sensitivity tests have high PPV in populations with a high prevalence of the disease. Initially, it was our intention to characterize the study population with negative HCV serology regarding demographic variables (age, gender, race, and schooling) and risk factors (transfusion of blood products, time and modality of dialysis, prior surgeries, injections in commercial drugstores, use of illicit drugs with needle sharing, tattoos, and piercings) for this infection in dialysis units, and to evaluate the reliability of the anti-HCV serology (EIA, 3rd generation), in relation to ALT and PCR, in the early detection of HCV infection in those patients. The panel study method was adapted for the analysis, since the demographic data did not present correlation with the results, and, among the risk factors, nosocomial exposure, which was common to all patients studied, was the most significant.

**ETHICAL ASPECTS OF THE STUDY**

This study was approved by the Research Ethics Committee, Internal Medicine Department, and by the Teaching and Research Department of the Hospital das Clínicas da Universidade Federal de Minas Gerais (UFMG). The objectives of this study were presented to dialysis units coordinators and their teams. They were also presented to all coordinators and study patients, who agreed to participate by signing an informed consent.

**RESULTS**

Forty-five (9%) of the 500 study patients were excluded during follow-up; the majority (27/60%) was excluded due to the lack of tests available for evaluation, followed by death (9/20%), kidney transplant (7/15.5%), and discharge from dialysis (2/4.4%), as shown in Figure 2. Based on statistical criteria described previously, this loss did not affect negatively the sample size. As for the demographic data, Table 2 shows a predominance of males (57.8%), and age above 40 (49%), of which 26.1% were older than 60 years. The study group had a mean age of 50 ± 15 years, ranging from 16 to 89 years. Non-Caucasian predominated (53%) over Caucasian (47%) patients. As for schooling, 286 patients (62%) did not finish junior high. End-stage renal disease is more prevalent in lower income populations

---

**Table 1** CHARACTERISTICS OF HEPATITIS C-DETECTION TESTS

<table>
<thead>
<tr>
<th>Tests</th>
<th>ALT</th>
<th>Anti-HCV</th>
<th>HCV RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>83**-90*</td>
<td>93**-100*</td>
<td>100** *</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>21**-91*</td>
<td>84**-99,92*</td>
<td>100** *</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>32**-57*</td>
<td>44**-99 *</td>
<td>100** *</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>97**-98*</td>
<td>98**- 100*</td>
<td>100** *</td>
</tr>
</tbody>
</table>

Reference values in IU/L: Immunocompetent Patients* (27-33) and with ESRD** (16-20), female and male, respectively.10,29,30

ALT sensitivity and specificity.10,16
Anti-HCV sensitivity and specificity.23
with less access to health care, lower schooling, and lower compliance with treatment of associated comorbidities. Hypertension is the most common cause of ESRD in Brazil, followed by diabetes mellitus, and glomerulopathies,3 which was also seen in the study population: hypertension, 62%, diabetes, 19%, and glomerulopathies, 13%.

Among the risk factors for hepatitis C investigated, blood transfusion had a higher prevalence: it affected 69.4% of the patients, although 60.2% were done after 1992, when serologic assays for HCV were available. As for the type of dialysis done previously, 80% reported that HD was the only modality, and 20% underwent PD before HD. Regarding treatment, 40.2% of the patients reported being treated in more than one dialysis unit, with a mean time of dialysis of 48.8 ± 41.2 months, ranging from 3 to 223 months. All patients underwent at least one surgical procedure: creation of an arteriovenous fistula for HD. The remainder risk factors for infection with HCV were not considered relevant in this patient population.

At the time of blood collection (month 0) for virological testing, 92% of the patients had normal ALT levels, and 7.9% had elevated ALT levels. Considering the higher reference value of 60% of the upper normal limit, as proposed by Gouvea et al. in 2004, 71.2% of the patients had normal ALT levels and 29% had elevated ALT levels. Anti-HCV serology was negative in all patients during the prospective three-month follow-up period, which showed a high NPV, i.e., 99.78%. Qualitative HCV RNA, the molecular biology test, was performed on blood samples of all 500 patients. Of those, only one (0.2%), a 52-year old Caucasian male with low schooling and hypertension-induced ESRD, without other risk factors, showed positive viremia during the follow-up period. This patient had not received any blood transfusions or undergone HD in another clinic, and had been on HD for 48 months at the time of the study. ALT levels were within reference levels, and he did not show seroconversion during the follow-up period, which only happened after 16

### Table 2  Demographic Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Categories</th>
<th>Frequency (n) / Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>263 (57.8)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>214 (47.0)</td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td>241 (53.0)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt; 20 years</td>
<td>4 (0.9)</td>
</tr>
<tr>
<td>20 – 39 years</td>
<td>109 (24.0)</td>
</tr>
<tr>
<td>40 – 59 years</td>
<td>222 (49.0)</td>
</tr>
<tr>
<td>&gt; 60 years</td>
<td>120 (26.1)</td>
</tr>
<tr>
<td>Schooling</td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>28 (6.2)</td>
</tr>
<tr>
<td>Incomplete Junior High</td>
<td>282 (62.0)</td>
</tr>
<tr>
<td>Junior High</td>
<td>48 (10.5)</td>
</tr>
<tr>
<td>Incomplete High School</td>
<td>12 (2.6)</td>
</tr>
<tr>
<td>High School</td>
<td>64 (14.1)</td>
</tr>
<tr>
<td>University Degree</td>
<td>21 (4.6)</td>
</tr>
<tr>
<td>Etiology of ESRD</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>192 (42.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>88 (19.0)</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>57 (13.0)</td>
</tr>
<tr>
<td>Interstitial nephropathy</td>
<td>30 (6.6)</td>
</tr>
<tr>
<td>Obstructive nephropathy</td>
<td>10 (2.2)</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>6 (1.3)</td>
</tr>
<tr>
<td>Other</td>
<td>29 (6.4)</td>
</tr>
<tr>
<td>Non identified</td>
<td>43 (9.5)</td>
</tr>
</tbody>
</table>
Table 3: ALT, Anti-HCV, and HCV RNA Results in 455 Patients with ESRD in HD Included in the Study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Categories</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT in relation to the URL</td>
<td>Normal</td>
<td>419</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>36</td>
<td>7.9</td>
</tr>
<tr>
<td>ALT in relation to 60% of URL</td>
<td>Normal</td>
<td>324</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>131</td>
<td>29</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>Negative</td>
<td>455</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HCV RNA</td>
<td>Negative</td>
<td>454</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*URL = Upper Reference Limit

weeks of positive viremia. Table 3 summarizes the results of biochemical, serologic, and molecular biology tests of the study population.

Discussion

Considering that the prevalence of hepatitis C in ESRD is high,1 especially in those on HD, whose higher susceptibility to infection can be attributed to changes in immune response,26,27 the nosocomial environment of dialysis units,6 the difficulty to diagnose this disorder due to normal or subtle elevation in ALT levels,9,10,16 the possibility of false-negative results,17,18 and the low viral load attributed, mainly, to dialysis and changes in immune response,21,23,24 we tried, in the present study, to evaluate the diagnostic methods for the early detection of hepatitis C and the periodicity that those tests should be performed in dialysis units.

Demographic aspects, such as age, gender, and race, the renal disease etiology, and schooling did not seem to correlate with a higher risk of transmission of HCV, as has been observed by some authors.8,15,22 Those data were collected in this study only to characterize study population and risk stratification. Therefore, the parameters of the sample population corresponded to the Brazilian population on dialysis, which is in agreement with the Diretrizes Brasileiras de Doença Renal Crônica, published in the Jornal Brasileiro de Nefrologia11 of the Sociedade Brasileira de Nefrologia (SBN) (Brazilian Guidelines on End-Stage Renal Disease, Brazilian Journal of Nephrology).

Most patients in this study were treated in only one dialysis unit, which suggests a lower vulnerability of this population to HCV infection, since patients who are treated in more than one dialysis unit have greater exposure to several virus genotypes.7,8,31,32

Risk factors for nosocomial HCV infection have been reviewed since the beginning of the 1990s, and both modality of treatment and time on dialysis have become more important since they show a direct relationship.2,7,8 This demonstrated the importance of universal precaution measures on the prevention of this infection. In the present study, mean time of dialysis was 48.8 months, but the incidence of positive viremia was low (0.2%). According to the characteristics of the study population, risk factors were not different of those found in HD, except for their close contact with other potential sources of nosocomial HCV transmission not detected by common hepatitis C markers. Thus, there are indications that HCV infection in this investigation occurred in the dialysis unit, since the only risk factors for HCV infection in the patient with positive viremia were time in HD and multiple sexual partners. However, this patient did not have other sexually transmitted diseases (HBV and HIV).

A positive correlation between ALT and HCV RNA was not observed, even after reducing ALT levels to 60% of the upper reference value.30 However, in patient with ESRD on dialysis, the upper reference value of ALT should be reduced considering that this marker is readily available in the public health system for disease detection and it can also indicate the need of serologic testing before the six-month period established in the last recommendation of the Ministry of Health about this subject.32 The sensitivity and specificity of this test is close to 97 and 99%, respectively, in immunocompetent patients, and false-negative results can be seen in patients with ESRD, which reduces sensitivity to 93% and specificity to 84%.10 An increase in its level may not be enough to detect HCV infection in the acute phase if the 12-week period for
seroconversion proposed in this study methodology is strictly followed.

The patient with positive HCV RNA in the present study had normal ALT levels if one considers its reference value. However, if one considers the level of 60%, this enzyme showed a small elevation in months - 3 and +3. But there did not seem to be a correlation between ALT levels and the presence of HCV infection, and further studies are necessary to evaluate this relationship. Although the size of the study population was significant, only one case of positive HCV RNA (0.2%) was observed, and it was not possible to correlate the prevalence of false-negative serologies with demographic data, risk factors, and ALT.

The methodology used in this investigation, i.e., quarterly evaluations of anti-HCV in historical and prospective results, was aimed at guaranteeing the inclusion of patients who were not in the immunological window. Therefore, the results of this investigation, i.e., a 0.2% prevalence of false-negative serology among 500 patients demonstrated the high sensitivity and high NPV of the anti-HCV test, corroborating its efficacy as a screening and follow-up test for patients on HD, and differing from other recent studies that demonstrated 0.8% to 2.3% prevalence of false-negative results. However, in those studies, the methodology of transversal cuts did not contemplate the analysis of historical or follow-up serologies of up to 12 weeks, which is considered the time necessary for seroconversion of patients infected recently. Thus, it is possible that the higher prevalence of negative anti-HCV reported by those studies was secondary to the inclusion of patients shortly before seroconversion.11,17

It was not the intention of this study to establish the traditional validation between anti-HCV and HCV RNA, the gold standard, for the diagnosis of hepatitis C by comparing a positive and a negative group. As for the NPV of the anti-HCV test, we assumed that individuals HCV RNA positive would also be anti-HCV positive (concordance close to 100%), i.e., presenting an elevated PPV, which is expected in high sensitivity tests in populations with a high prevalence of the disease. Therefore, one can say that a NPV for this population was identified (99.78%), since serologic tests with high sensitivity and specificity were used, similar to the results reported by other studies.10,18,21,22

The only patient with a false-negative serology remained so during the 3-month follow-up period, and HCV seroconversion was observed 16 weeks after the first positive viremia. Therefore, it was demonstrated that the anti-HCV test has high specificity and NPV. Even with only one false-negative patient, the importance of adopting strict universal precaution measures should be emphasized, since, based on routine tests performed at dialysis units, this patient remained a potential source of HCV infection for 16 weeks.

**CONCLUSION**

The results observed in this study can be attributed to the methodology used, which aimed at detecting patients in the so called immunological window. ALT levels did not contribute for the early diagnosis of acute hepatitis C, confirming its low sensitivity and specificity in the diagnosis of acute hepatitis C in patients with ESRD on HD. Routine HCV RNA should not precede a critical analysis of the interval between serologic tests, which are recommended every six months, for the early detection of seroconversion. An association between risk factors and positive HCV serology could not be evaluated since only one patient had a positive HCV RNA. Due to the risk of false-negative serology, the strict observation of universal precaution measures in dialysis units is fundamental to prevent carriers of HCV from disseminating the infection before being aware of their state of potential sources of infection.

We concluded that the reduction in the interval between anti-HCV tests, the use of a lower ALT cut-off, and the compliance with strict universal precaution measures to control the nosocomial transmission of HCV could contribute for the early detection and control of HCV infection in patients with ESRD on HD. Further studies with standard tests to detect hepatitis C are necessary to evaluate the most adequate interval for serologic tests.

**ACKNOWLEDGEMENTS**

We would like to thank the participation of the Research Support Nucleus and the Central Laboratory of the Faculdade de Medicina at UFMG, the Dialysis Units of Belo Horizonte, the Paula Castro Laboratory, and the financial support of FAPEMIG/CNPq.

**REFERENCES**


