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Diagnostic Discordance for Hepatitis C Virus Infection in Hemodialysis Patients

Kamyar Kalantar-Zadeh, MD, PhD, MPH, Loren G. Miller, MD, MPH, and Eric S. Daar, MD

**Background:** Hepatitis C virus (HCV) infection is associated with an increase in proinflammatory cytokine levels. Similar changes are seen in maintenance hemodialysis patients with malnutrition-inflammation-cachexia syndrome (MICS), which is associated with poor clinical outcomes in this population. We hypothesized that HCV transcription-mediated amplification (TMA), a sensitive qualitative molecular test for HCV RNA, may identify maintenance hemodialysis patients with HCV infection not detected by means of antibody enzyme immunoassay (EIA), particularly in those with MICS. **Methods:** We evaluated HCV status in 314 maintenance hemodialysis patients by using HCV antibody EIA (version 2.0; Abbott Laboratories, Abbott Park, IL) and HCV TMA (Bayer Diagnostics Laboratories, Berkeley, CA). **Results:** Twenty-five patients (8%) were EIA positive (EIA+/TMA-); 4 patients (1%), EIA+/TMA negative (TMA-), and 22 patients (7%), EIA-/TMA+. In the 47 TMA+ patients, the sensitivity of EIA for HCV infection was only 53%. TMA+ patients had lower albumin levels and higher tumor necrosis factor α and serum glutamic oxaloacetic transaminase levels than TMA- patients. EIA+/TMA+ patients were more likely than EIA-/TMA- or EIA+/TMA- patients to have hypoalbuminemia and higher iron and transaminase levels. Of all TMA+ patients, EIA- patients were more likely to have diabetes, be on dialysis therapy longer, and have lower liver enzyme levels and higher proinflammatory cytokine levels, including tumor necrosis factor α and interleukin 6. **Conclusion:** Maintenance hemodialysis patients infected with HCV according to TMA have clinical features suggestive of MICS. In this population, HCV EIA appears to have a low sensitivity for the identification of HCV infection, which may be caused by the confounding effect of MICS or other demographic or clinical factors. These apparently false-negative HCV antibody test results are seen in persons with a longer time on hemodialysis therapy, mirroring observations in other populations with serious progressive conditions, such as human immunodeficiency virus infection. Am J Kidney Dis 46:290-300.

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INDEX WORDS: Hepatitis C virus (HCV); hemodialysis (HD) patient; transcription-mediated amplification; enzyme immunoassay; malnutrition-inflammation-cachexia syndrome; proinflammatory cytokines.

**H**epatitis C virus (HCV) infection, the most common cause of chronic liver disease in the United States, is particularly common in patients with chronic kidney disease, especially those undergoing maintenance hemodialysis (MHD). MHD patients are at high risk for acquiring HCV infection from transfusion of blood products or other parenteral exposures to HCV during MHD treatments. In the majority of these patients, HCV infection is not cleared and results in chronic infection with HCV viremia. In non-MHD populations, HCV infection typically is associated with the development of antibodies to HCV, with chronic infection defined as...
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sensitivity in detecting HCV infection. Iden-
questioned as having inadequate accuracy and
Routine serological tests for HCV have been
infected MHD patients are poorly described.
Unfortunately, clinical and laboratory features of HCV-
iinfecting HCV diagnostic tests.

cation of HCV infection, determined by means of
sensitive molecular assay that detects HCV
RNA, and HCV antibody enzyme immunoas-
say (EIA), the commonly used serological screen-
ing test. To that end, we examined whether
elements of MICS were associated with discord-
ant results between these 2 tests. Because previ-
ous investigations suggested that such molecular
tests as polymerase chain reaction (PCR) were
more sensitive for diagnosing HCV infection
than antibody EIA, we hypothesized that TMA
would detect additional HCV-infected patients
compared with EIA, which could be more sensi-
tive to confounding effects of MICS. We also
studied demographic, clinical, and laboratory
characteristics of MHD patients, including mark-
ers of MICS, stratified by results of the 2 men-
tioned HCV diagnostic tests.

METHODS

Patient Population
Subjects participating in the Nutritional and Inflammatory
Evaluation in Dialysis (NIED) Study originated from a pool
of approximately 1,300 MHD outpatients in 8 DaVita Inc
dialysis facilities in the South Bay Los Angeles area (see
NIED Study Web site at www.NIEDStudy.org for more
details, as well as previous publications). Inclusion
criteria were outpatients who had been undergoing MHD
therapy for at least 8 weeks, were 18 years or older, and had
signed a local institutional review board–approved consent
form. Patients with an anticipated life expectancy of less
than 6 months (for example, because of a metastatic malign-
cy or advanced human immunodeficiency virus disease)
were excluded. In the NIED Study cohort, no patient cur-
rently is known to have a positive human immunodeficiency
virus serological test result. In the midphase of the NIED
Study (second half of 2003), 360 MHD patients from 8
dialysis units actively attended the study. During this visit,
blood samples were obtained from 351 of these individuals,
with 9 individuals not present in the dialysis units at the time
of blood drawing. After all planned laboratory measure-
ments were performed, 314 specimens remained for retro-
spective HCV testing.

Laboratory Evaluation
Predialysis blood samples and postdialysis serum urea
nitrogen values were obtained on a midweek day and coin-
cided chronologically with the quarterly blood tests of
DaVita facilities. Single-pool Kt/V was used to represent
weekly dialysis dose. All routine laboratory measurements
were performed by DaVita Laboratories (Deland, FL) by
using automated methods, and average values for each
laboratory test within the 13-week study period were calcu-
lated and used for data analyses in this study.

Serum C-reactive protein and 2 proinflammatory cyto-
kines, interleukin 6 (IL-6) and tumor necrosis factor α
(TNF-α), were measured as indices of degree of inflammation. High-sensitivity C-reactive protein was measured by means of a turbidimetric immunoassay in which a serum sample is mixed with latex beads coated with antihuman C-reactive protein antibodies, forming an insoluble aggregate (WPI, Osaka, Japan; measured in milligrams per liter; normal range, <3.0 mg/L). High-sensitivity IL-6 and TNF-α immunoassay kits based on a solid-phase sandwich enzyme-linked immunosorbent assay using recombinant human IL-6 and TNF-α were used to measure serum proinflammatory cytokines (R&D Systems, Minneapolis, MN; measured in picograms per milliliter; normal range: IL-6, <9.9 pg/mL; TNF-α, <4.7 pg/mL). C-Reactive protein and cytokines were measured in the General Clinical Research Center Laboratories of Harbor-UCLA Medical Center. Serum prealbumin was analyzed by means of an antigen-antibody complex assay, and total homocysteine concentrations were determined by means of high-performance liquid chromatography at Harbor-UCLA Clinical Laboratories.

HCV Assays

Two tests for the detection of HCV infection were performed: (1) HCV antibody EIA (version 2.0; Abbott Laboratories, Abbott Park, IL) and (2) TMA (Versant HCV; Bayer Diagnostics Laboratories, Berkeley, CA). For the latter test, 3 steps were performed in a single tube, including target capture, target amplification by isothermal TMA, and detection of amplified product by hybridization and dual kinetic assays. In the final step, amplicons were detected by using complementary-single stranded probes labeled with a chemiluminescent tag. Chemiluminescent signal was read as relative light units, and data were reported as both calculated relative light units and signal-cutoff ratios. The specimen was considered reactive, or having detectable HCV RNA, when the signal-cutoff ratio was greater than 1. The technology and performance of the assay have been well described previously. TMA is considered the gold standard for HCV diagnosis given the impracticality and risk of using liver biopsy as a diagnostic test and because a positive diagnostic molecular test result for HCV is considered sufficient evidence to warrant liver biopsy for the purpose of treatment. 18-21 TMA was considered reactive, or having detectable HCV RNA, when the signal-cutoff ratio was greater than 1. A gold standard for HCV diagnosis is the imprecision and risk of using liver biopsy as a diagnostic test and because a positive diagnostic molecular test result for HCV is considered sufficient evidence to warrant liver biopsy for the purpose of treatment. 18-21 TMA was used to detect significant differences between continuous variables in 2 or more groups, respectively. Chi-square and Kruskal-Wallis rank tests were used for categorical variables. Sensitivity is defined as the proportion of positive tests in HCV-infected MHD patients. TMA was used as the gold standard for HCV diagnosis. Specificity is the proportion of negative tests in non-HCV-infected patients. Multivariate logistic regression models were fitted to construct the odds ratio of HCV infection, controlling for confounding covariates. Fiducial limits are given as mean ± SD. For such non-normally distributed measures as inflammatory markers, both natural (untransformed) and logarithmic values were examined. P less than 0.05 or a 95% confidence interval (CI) that did not span 1.00 is considered statistically significant. P between 0.05 and 0.10 is considered borderline significant. Descriptive and multivariate statistics were carried out using the statistical software Stata, version 7.0 (Stata Corp, College Station, TX).

RESULTS

Of 314 MHD patients who were tested by means of both EIA and TMA, 263 individuals (84%) were HCV+ using both tests (Fig 1). Twenty-five MHD patients (8%) were found to be HCV+ according to both tests, including 6 patients who had had previous positive EIA test results in their dialysis clinics when dialysis charts were reviewed retrospectively, whereas 22 patients (7%) were TMA+ but EIA− and 4 patients (1%) were TMA− but EIA+. None of the latter 2 groups and none of the rest of the cohort were known previously to have HCV infection. Table 1 lists the tabular description of these results. If TMA is considered the gold (reference) standard, EIA is 99% specific, but has a low sensitivity (53%) in this population, i.e., almost half the HCV-infected MHD patients were missed by using antibody EIA testing.

Table 2 lists baseline demographic, clinical, and laboratory characteristics of the cohort based on the detection of HCV RNA by means of the TMA assay. African Americans comprised almost half of all HCV TMA+ subjects (47%) compared with almost one quarter in HCV RNA− patients (27%). HCV RNA+ subjects had significantly lower serum albumin concentrations with increased total protein levels compared with HCV RNA− patients. Levels of serum glutamic oxaloacetic transaminase, also known as aspartate aminotransferase (AST), were higher in TMA+ MHD patients, as were TNF-α levels.

Three groups of MHD patients stratified according to HCV TMA and EIA results, ie, TMA−/EIA− (n = 263), EIA+/TMA− (n = 25), and EIA+/TMA+ (n = 22) are compared in Table 3. The other discordant group (TMA−/EIA+) included only 4 patients and hence was not included in analyses. Black patients were significantly overrepresented in the EIA+/TMA+ group (52%). EIA+/TMA+ patients had been undergoing dialysis therapy for a longer time than the other groups and more than two thirds had diabetes mellitus, whereas the prevalence of diabetes
in concordant (EIA⁺/TMA⁺) patients was only 40% (P = 0.02). Moreover, they had significantly higher IL-6 levels than EIA⁺/TMA⁻ patients. EIA⁺/TMA⁻ patients also had significantly lower serum albumin concentrations and higher total protein, AST, alkaline phosphatase, and iron levels than the other groups. Conversely, serum IL-6 levels were significantly greater in the EIA⁻/TMA⁺ group compared with the 2 other groups, whereas TNF-α levels were similar in both TMA⁺ groups, with both significantly greater than in TMA⁻ subjects.

In multivariate logistic regression models, the association between demographic, clinical, and laboratory characteristics and risk for HCV positivity was examined. Table 4 lists multivariate adjusted odds ratios of HCV positivity based on each of the TMA or EIA test results, as well as the odds ratio of EIA positivity among all TMA⁻ patients. In both the TMA⁺ and EIA⁺ models, black race, lower serum albumin level, higher AST level, and higher iron level were associated with HCV positivity. A low body mass index was associated with TMA positivity. Diabetes mellitus showed a trend toward an association with TMA positivity. Among inflammatory markers, a higher TNF-α level was associated with 2.41 higher odds of EIA positivity.

DISCUSSION

In a cross-sectional analysis of 314 MHD patients, we show that the traditional HCV EIA antibody screening test is insensitive for detecting HCV infection. Using a molecular test for HCV, the qualitative HCV TMA assay, as the gold standard, HCV EIA appears to miss almost half of all HCV-infected MHD patients. Although this discordance has been described before,¹² we present an unusually high degree of it for the first time in any population.

Moreover, we found that EIA⁺ patients were more likely to have signs of protein-energy malnutrition (low serum albumin and prealbumin
levels) compared with EIA− patients, as well as increased liver enzyme and iron levels. TMA+ patients tended to show higher serum proinflammatory cytokine levels than TMA− patients. In addition, TMA+/EIA− patients included a significantly greater proportion with diabetes and tended to have undergone chronic dialysis treatment for a longer time. These differences may have a role in distinguishing between chronically HCV-infected (TMA+/H11001) patients who are antibody EIA− versus EIA+. Hence, observed differences might explain and predict why some HCV-infected MHD patients are EIA−. Consequently, the observed differences between these groups, such as greater prevalence of diabetes or longer dialysis vintage time, may allow for targeting HCV EIA− patients who might need additional molecular HCV diagnostic testing.

Chronic HCV infection is common in MHD patients, but its exact prevalence is not clear.1-4,7,36 A recent survey from the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, indicated that in 2000, the prevalence of HCV antibody positivity was 8.4% in 135,599 patients, representing 58% of US outpatient dialysis clinics that performed screening.10 Recently, we analyzed a national dialysis database of more than 37,000 MHD patients and found that the HCV EIA test was performed in only 2,778 individuals during the selected calendar quarter.5 In this retrospective study, 363 MHD patients (13%) were EIA−. However, these estimates are based solely on results of antibody testing. Other investigations have shown that molecular-based testing for HCV RNA, such as PCR and TMA, is more sensitive than EIA testing in MHD patients.12-14,37,38 Therefore, the true preva-

### Table 2. Demographic, Clinical, and Laboratory Characteristics According to Detection of HCV RNA by Means of TMA Test in 314 MHD Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>TMA−</th>
<th>TMA+</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (% of total)</td>
<td>267 (85)</td>
<td>47 (15)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>48</td>
<td>49</td>
<td>0.9</td>
</tr>
<tr>
<td>Ethnicity (% Hispanic)</td>
<td>53</td>
<td>40</td>
<td>0.11</td>
</tr>
<tr>
<td>Race (% black)</td>
<td>27</td>
<td>47</td>
<td>0.007</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>47</td>
<td>53</td>
<td>0.5</td>
</tr>
<tr>
<td>Age (y)</td>
<td>54.3 ± 15</td>
<td>52.6 ± 14</td>
<td>0.5</td>
</tr>
<tr>
<td>Dialysis vintage (mo)</td>
<td>38 ± 35</td>
<td>35 ± 38</td>
<td>0.5</td>
</tr>
<tr>
<td>Dialysis vintage &gt;1 y (%)</td>
<td>78</td>
<td>70</td>
<td>0.22</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>3.95 ± 0.29</td>
<td>3.86 ± 0.26</td>
<td>0.04</td>
</tr>
<tr>
<td>Prealbumin (mg/dL)</td>
<td>29 ± 7</td>
<td>28 ± 8</td>
<td>0.25</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.0 ± 0.4</td>
<td>7.2 ± 0.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>145 ± 33</td>
<td>138 ± 31</td>
<td>0.17</td>
</tr>
<tr>
<td>C-Reactive protein (mg/L)</td>
<td>4.9 ± 3.2</td>
<td>4.5 ± 3.4</td>
<td>0.5</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>15.0 ± 42.3</td>
<td>21.5 ± 63.8</td>
<td>0.4</td>
</tr>
<tr>
<td>TNF-α (mg/L)</td>
<td>7.4 ± 4.9</td>
<td>9.7 ± 9.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Total homocysteine (μmol/L)</td>
<td>26 ± 9</td>
<td>28 ± 12</td>
<td>0.3</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>17 ± 10</td>
<td>25 ± 14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>114 ± 54</td>
<td>127 ± 47</td>
<td>0.13</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.9 ± 1.3</td>
<td>5.9 ± 1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Intact parathyroid hormone (pg/mL)</td>
<td>353 ± 307</td>
<td>376 ± 345</td>
<td>0.6</td>
</tr>
<tr>
<td>Total iron-binding capacity (mg/dL)</td>
<td>202 ± 31</td>
<td>209 ± 34</td>
<td>0.15</td>
</tr>
<tr>
<td>Iron (mg/mL)</td>
<td>66 ± 17</td>
<td>73 ± 24</td>
<td>0.02</td>
</tr>
<tr>
<td>Iron saturation ratio (%)</td>
<td>33 ± 8</td>
<td>35 ± 9</td>
<td>0.06</td>
</tr>
<tr>
<td>Ferritin (mg/mL)</td>
<td>633 ± 297</td>
<td>635 ± 357</td>
<td>0.9</td>
</tr>
<tr>
<td>Erythropoietin (U/wk)</td>
<td>13,589 ± 8,519</td>
<td>14,122 ± 12,455</td>
<td>0.7</td>
</tr>
<tr>
<td>Blood hemoglobin (g/dL)</td>
<td>12.1 ± 0.6</td>
<td>12.1 ± 0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Lymphocytes (% of white blood cells)</td>
<td>23 ± 7</td>
<td>24 ± 7</td>
<td>0.4</td>
</tr>
<tr>
<td>White blood cells (1,000/high-power field)</td>
<td>7.1 ± 1.7</td>
<td>6.6 ± 1.4</td>
<td>0.06</td>
</tr>
</tbody>
</table>

NOTE. Count values expressed as percentage, continuous values expressed as mean ± SD. To convert albumin, protein, and hemoglobin in g/dL to g/L, multiply by 10; cholesterol in mg/dL to mmol/L, multiply by 0.02586; phosphorus in mg/dL to mmol/L, multiply by 0.3229; ferritin in ng/mL to μg/L, multiply by 1.
Table 3. Demographic, Clinical, and Laboratory Characteristics Based on HCV Antibody EIA and HCV RNA by TMA Status

<table>
<thead>
<tr>
<th>Variable</th>
<th>No HCV Infection (EIA+/TMA−)</th>
<th>TMA− Only* (EIA−/TMA−)</th>
<th>Both HCV Tests Positive* (EIA+/TMA+)</th>
<th>Analysis of Variance</th>
<th>t-Test* P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (%)</td>
<td>263 (85)</td>
<td>22 (7)</td>
<td>25 (8)</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>48</td>
<td>50</td>
<td>48</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Ethnicity (% Hispanic)</td>
<td>53</td>
<td>50</td>
<td>32</td>
<td>0.14</td>
<td>0.10</td>
</tr>
<tr>
<td>Race (% black)</td>
<td>28</td>
<td>41</td>
<td>52</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>47</td>
<td>68</td>
<td>40</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>Age (y)</td>
<td>54.2 ± 15</td>
<td>54.2 ± 14.6</td>
<td>51.2 ± 13.6</td>
<td>0.6</td>
<td>0.23</td>
</tr>
<tr>
<td>Dialysis vintage (mo)</td>
<td>39 ± 35</td>
<td>46 ± 45</td>
<td>24 ± 26</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>Dialysis vintage &gt; 1 y (%)</td>
<td>78</td>
<td>81</td>
<td>58</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>3.95 ± 0.26</td>
<td>3.93 ± 0.25</td>
<td>3.79 ± 0.29</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Prealbumin (mg/dL)</td>
<td>29 ± 7</td>
<td>31 ± 10</td>
<td>24 ± 6</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>Total protein (mg/dL)</td>
<td>7.0 ± 0.4</td>
<td>6.9 ± 0.4</td>
<td>7.4 ± 0.6</td>
<td>0.005</td>
<td>0.003</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>144 ± 33</td>
<td>143 ± 22</td>
<td>133 ± 38</td>
<td>0.24</td>
<td>0.13</td>
</tr>
<tr>
<td>C-Reactive protein (mg/L)</td>
<td>4.9 ± 3.2</td>
<td>4.9 ± 3.6</td>
<td>4.2 ± 3.2</td>
<td>0.60/0.5†</td>
<td>0.24/0.21†</td>
</tr>
<tr>
<td>IL-6 (mg/L)</td>
<td>15.0 ± 42.6</td>
<td>28.3 ± 49.7</td>
<td>15.5 ± 19.0</td>
<td>0.4/0.01†</td>
<td>0.24/0.01†</td>
</tr>
<tr>
<td>TNF-α (mg/L)</td>
<td>7.4 ± 5.0</td>
<td>9.7 ± 12.2</td>
<td>9.6 ± 7.0</td>
<td>0.05/0.01†</td>
<td>0.5/0.9†</td>
</tr>
<tr>
<td>Total homocysteine (µmol/L)</td>
<td>26 ± 9</td>
<td>30 ± 17</td>
<td>26 ± 6</td>
<td>0.24</td>
<td>0.16</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>17 ± 10</td>
<td>18 ± 9</td>
<td>31 ± 16</td>
<td>&lt;0.0001</td>
<td>0.0007</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>114 ± 54</td>
<td>111 ± 30</td>
<td>140 ± 55</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.9 ± 1.3</td>
<td>5.8 ± 1.2</td>
<td>5.9 ± 1.6</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Intact parathyroid hormone (U/L)</td>
<td>355 ± 309</td>
<td>370 ± 245</td>
<td>381 ± 418</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Total iron-binding capacity (mg/dL)</td>
<td>201 ± 31</td>
<td>201 ± 32</td>
<td>216 ± 34</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Iron (ng/mL)</td>
<td>66 ± 17</td>
<td>62 ± 10</td>
<td>84 ± 28</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Iron saturation ratio (%)</td>
<td>33 ± 8</td>
<td>31 ± 5</td>
<td>38 ± 10</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>636 ± 297</td>
<td>597 ± 310</td>
<td>669 ± 397</td>
<td>0.7</td>
<td>0.24</td>
</tr>
<tr>
<td>Erythropoietin dose (U/wk)</td>
<td>13,677 ± 8,547</td>
<td>11,896 ± 4,758</td>
<td>16,080 ± 16,401</td>
<td>0.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Blood hemoglobin (g/dL)</td>
<td>12.1 ± 0.6</td>
<td>12.1 ± 0.5</td>
<td>12.1 ± 0.8</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Peripheral lymphocytes (% white blood cells)</td>
<td>23 ± 7</td>
<td>23 ± 6</td>
<td>25 ± 7</td>
<td>0.5</td>
<td>0.18</td>
</tr>
<tr>
<td>White blood cells (/high-power field)</td>
<td>7.1 ± 1.7</td>
<td>6.8 ± 1.4</td>
<td>6.4 ± 1.5</td>
<td>0.12</td>
<td>0.21</td>
</tr>
</tbody>
</table>

NOTE. All nonpercentage values are expressed as mean ± SD. Analysis of variance compares all 3 groups, whereas t-test pertains to the last 2 groups. To convert albumin, protein, and hemoglobin in g/dL to g/L, multiply by 10; cholesterol in mg/dL to mmol/L, multiply by 0.02586; phosphorus in mg/dL to mmol/L, multiply by 0.3229; ferritin in ng/mL to µg/L, multiply by 1.

* t-Test compares discordant cases with those in which both test results were positive.
† Second P pertains to analyses based on logarithmic transformation of individual values.

Hepatitis C infection may be much greater than the CDC estimates. Consistent with the CDC investigation, in our study, we found a point prevalence of HCV infection by means of EIA to be 9%. However, HCV RNA testing by means of TMA detected a 15% prevalence in our population (47 of 314 patients), including HCV viremia in all except 4 EIA+ patients (1%). EIA+/TMA− patients most likely represent those who were HCV infected and then cleared infection or false-positive antibody EIA results. However, given that some patients with HCV infection are only intermittently viremic, it is still possible that these 4 EIA+/TMA− patients are truly HCV infected. In a recent study by Sypsa et al, sequential serum samples from 562 MHD patients in Greece were tested by means of EIA to examine the HCV seroconversion incidence. However, in this study, HCV RNA was performed retrospectively and only on patients found to be EIA+. Although the investigators concluded that a wide window period of HCV infection in MHD patients existed, longitudinal studies
with uniform HCV RNA testing for all members of the cohort are needed to answer these questions.

Assuming that all EIA results were true positives, the clearance rate would be 4 of 51 (51 being all with evidence of infection by means of EIA or TMA). This 7.8% rate of HCV clearance would be relatively low compared with such distinctly different patient populations as children or young adults, but consistent with these subjects having immune defects resulting in a greater frequency of HCV chronicity, such as human immunodeficiency virus–infected subjects. In our study, TMA patients who had diabetes and had undergone dialysis therapy for a significantly longer time were more likely to be EIA−, which may indicate the greater likelihood of immune system dysfunction. However, given that some patients with HCV infection are only intermittently viremic, it still is possible that these 4 EIA+/TMA− patients were truly HCV infected. Finally, in this latter group, a false-positive EIA result may explain the results. Longitudinal studies to follow up these patients over time are needed to answer these questions. It also is important to note that the NIED Study cohort currently does not include human immunodeficiency virus–positive MHD patients.

Among liver transaminases, we measured AST, whereas serum alanine aminotransferase was not measured in our study, as is the case in the majority of dialysis clinics across the United States. Liver function test results, such as AST or alanine aminotransferase levels, may be elevated only mildly to moderately, if at all, in HCV-infected MHD patients, although a newly elevated alanine aminotransferase level may be somewhat more specific, but not adequately sensitive, for the detection of chronic HCV infection in this population.

Table 4. Multivariate Adjusted Odds Ratios for Selected Clinical and Laboratory Variables in Relation to HCV Positivity in 314 MHD Patients

<table>
<thead>
<tr>
<th>Variable (direction and magnitude of change)</th>
<th>Odds Ratio (95% CI)</th>
<th>P</th>
<th>Odds Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (each 10-y decrease)</td>
<td>1.31 (0.99-1.73)</td>
<td>0.06</td>
<td>1.21 (0.84-1.73)</td>
<td>0.3</td>
</tr>
<tr>
<td>Female (v male)</td>
<td>1.16 (0.57-2.37)</td>
<td>0.7</td>
<td>1.26 (0.49-3.27)</td>
<td>0.6</td>
</tr>
<tr>
<td>Black (v other races)</td>
<td>3.75 (1.76-7.97)</td>
<td>0.001</td>
<td>3.93 (1.43-10.81)</td>
<td>0.008</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.05 (0.91-4.63)</td>
<td>0.08</td>
<td>0.71 (0.24-2.09)</td>
<td>0.5</td>
</tr>
<tr>
<td>Dialysis vintage ≥ 12 mo</td>
<td>0.80 (0.37-1.75)</td>
<td>0.6</td>
<td>0.67 (0.24-1.84)</td>
<td>0.4</td>
</tr>
<tr>
<td>Body mass index (each 1-k/m² decrease)</td>
<td>1.09 (1.02-1.17)</td>
<td>0.01</td>
<td>1.09 (0.99-1.20)</td>
<td>0.07</td>
</tr>
<tr>
<td>AST (each 10-U/L increase)</td>
<td>1.45 (1.08-1.94)</td>
<td>0.01</td>
<td>1.51 (1.04-2.18)</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum albumin (each 0.1-g/dL decrease)</td>
<td>1.15 (0.99-1.33)</td>
<td>0.06</td>
<td>1.34 (1.10-1.63)</td>
<td>0.004</td>
</tr>
<tr>
<td>Serum iron (each 10-ng/mL increase)</td>
<td>1.19 (0.99-1.43)</td>
<td>0.07</td>
<td>1.52 (1.18-1.94)</td>
<td>0.001</td>
</tr>
<tr>
<td>Log C-reactive protein (each 1-unit increase)</td>
<td>0.83 (0.50-1.37)</td>
<td>0.5</td>
<td>0.67 (0.34-1.31)</td>
<td>0.24</td>
</tr>
<tr>
<td>Log IL-6 (each 1-unit increase)</td>
<td>1.05 (0.68-1.63)</td>
<td>0.8</td>
<td>1.26 (0.74-2.16)</td>
<td>0.4</td>
</tr>
<tr>
<td>Log TNF-α (each 1-unit increase)</td>
<td>1.69 (0.91-3.15)</td>
<td>0.10</td>
<td>2.41 (1.06-5.47)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

NOTE. P > 0.25 expressed with 1 decimal. In the middle column, HCV positivity is defined as HCV RNA test (TMA) positive; in the last (right) column, this definition is based on EIA test exclusively. The reference population in each group consists of MHD patients with a negative test result. To convert albumin in g/dL to g/L, multiply by 1.

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sensitive than PCR. Several studies showed the superiority and reliability of HCV TMA in detecting HCV infection in the general population compared with HCV PCR. Krajden et al examined 300 specimens, including 112 samples that were indeterminate by means of EIA, but PCR−, and 79 samples that were EIA+ and PCR− showed that TMA was more sensitive than PCR, required less time for test result completion, and had a greater throughput. Comanor et al examined 97 patients treated for HCV in whom HCV RNA was not detected by means of PCR. TMA detected HCV RNA in 27 samples (34.6%) at the end of therapy and 76 follow-up samples (97.4%) from relapsing patients, but not in any end-of-therapy or follow-up samples from sustained responders.

To our knowledge, TMA was tested in MHD patients in only 1 previous investigation. Khan et al examined both TMA and PCR in 80 EIA+ and 100 EIA− MHD patients who were undergoing a kidney transplantation evaluation workup. In their study, 11 EIA+ patients had negative PCR results, but positive TMA results, whereas only 2 patients had the opposite concordant status. Among EIA− patients, only 5 patients were TMA+ (including 2 patients with PCR+ results). They concluded that TMA identified active HCV infection in more EIA+ and EIA− patients than PCR. The TMA positivity prevalence in EIA+ patients (5%) was similar to the 7% found in our study.

There are strengths to our investigation. First, the sample size is large and includes many individuals with diabetes mellitus. Second, unlike previous cohorts, our cohort has been extensively characterized for markers of inflammation and nutrition. The availability of these measures allowed us to show that HCV infection independently influences these markers in a group of MHD patients and that selected markers are associated with and may explain why selected individuals are HCV viremic while antibody negative. Third, patients in this cohort were selected randomly without having prior knowledge of their HCV status. Finally, the same blood specimens used to measure markers of MICS also were used for the 2 HCV tests, ie, TMA and EIA.

Our current study may be limited by selection bias. During recruitment in 8 dialysis units (with >1,200 MHD patients), it is possible that only MHD patients who were generally healthier and more health conscious and had better nutritional status agreed to participate (360 patients). Hence, MHD patients might include disproportionately fewer sick and better nourished individuals. The annual mortality rate in all patients of the study dialysis units was 15%, whereas it was as low as 10% in selected patients for the NIED Study. However, selection bias with such a direction generally would lead to a bias toward the null, so without this bias, our positive results probably would have been even stronger and the associations would have been more prominent. Another possible limitation is that EIA had false negativity because of technical constraints. This concern led us to retest all HCV EIA+/TMA− patients. Repeated testing yielded concordant (negative) results consistent with our initial findings. Moreover, false positivity of TMA results, although a possibility, would be an unlikely explanation for most patients given the previously reported specificity of this assay. In addition, there were some unique associations between EIA−/TMA− subjects and inflammatory markers, such as significantly increased IL-6 and TNF-α levels compared with EIA+/TMA− patients (Table 3). The reason that liver enzyme levels were greater in TMA+ patients who also were EIA+ is less clear. Although this could be consistent with TMA false-positive results, differences in the patient population and immunologic competence of those able to maintain an immunologic response to HCV may be a more likely explanation. Although an insufficient sample was available for confirmatory testing by means of an alternative molecular method for detection of HCV RNA, especially in the discordant EIA+/TMA+ group, our prevalence of TMA positivity in EIA− patients of 7% was similar to the 5% found by Khan et al in their population of pretransplantation MHD patients.

In conclusion, we found that EIA antibody testing for HCV infection in MHD patients is associated with a high proportion of false-negative results. This suggests that assays for the detection of HCV RNA, such as TMA or PCR, may be an important tool for diagnosing unrecognized HCV infection in this population. In addition, we show that selected groups are missed by means of antibody EIA; namely, those with dia-
betes mellitus and a longer time on dialysis therapy. The mechanism behind this observation may be further related to the observed association to elevations in levels of inflammatory cytokines, particularly IL-6. Because HCV-infected MHD patients may be a source of infection to others, including dialysis clinic staff and other outpatients, dialysis clinics should be well equipped with modalities to detect new HCV infections. Because EIA is the most commonly used method to evaluate HCV infection in the MHD population and much of our knowledge of HCV infection in this population is based on this limited test, molecular-based, rather than antibody-based, screening for this infection strongly needs consideration. Our observed EIA/TMA discrepancy for HCV infection detection also should be considered in the context of other discrepancies, including that between EIA and PCR, as well as other clinical settings, such as renal transplantation. Hence, such molecular tests as TMA may be the preferred screening test for the diagnosis of HCV infection in renal transplant recipients. Given our observed relationship between HCV infection and MICS and the association between levels of detrimental cytokines and HCV infection in this population, further investigation is needed to define whether these markers explain the greater mortality seen in this population.

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