Dialysis Therapies

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.
© *2005 by the National Kidney Foundation, Inc.*

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

HEPATITIS 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).[1-10] 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.[6] In non-MHD populations, HCV infection typically is associated with the development of antibodies to HCV, with chronic infection defined as

persistence of HCV RNA. Consequently, HCV antibody testing generally is used as a screening tool for evidence of infection, with virological tests for HCV RNA used to establish evidence of persistence.[11] Patients with chronic kidney disease with persistent HCV infection may develop serious and progressive chronic liver disease, with its associated long-term morbidity and mortality related to cirrhosis and hepatocellular carcinoma.[6]

The mean prevalence of antibodies to HCV in the US dialysis population in 2000 has been estimated to be 8.4%.[10] Other investigations of this population have shown a prevalence of HCV infection between 5% and 20%.[1-5] However, given that serological tests for HCV infection may miss some human immunodeficiency virus infections,[12-14] the exact prevalence of HCV infection in MHD patients, who total more than a quarter of a million persons, is not known. Unfortunately, clinical and laboratory features of HCV-infected MHD patients are poorly described. Routine serological tests for HCV have been questioned as having inadequate accuracy and sensitivity in detecting HCV infection.[12-14] Identifying HCV-infected MHD patients is vital to better understand the natural history of infection in these patients and evaluating and counseling them regarding the potential role of HCV treatment and renal transplantation.

In MHD patients, a strong association between the elements of malnutrition-inflammation-cachexia syndrome (MICS) and poor clinical outcome has been observed.[15,16] Dialysis treatment is characterized by a variety of changes in metabolic parameters, including increases in proinflammatory cytokine levels.[15] These increases in cytokine levels are remarkably similar to changes observed in patients with chronic HCV infection.[17-19] HCV may intensify oxidative stress in patients with uremia, leading to adverse cardiovascular outcomes.[19] Hence, HCV infection may be associated with a greater mortality rate in MHD patients, and if so, the link may be through MICS or independently through other mechanisms.[5] Therefore, better understanding of the possible link between HCV infection and MICS has major clinical relevance in this population.

In this investigation, we compared the prevalence of HCV infection, determined by means of transcription-mediated amplification (TMA), a sensitive molecular assay that detects HCV RNA,[12] and HCV antibody enzyme immunoassay (EIA), the commonly used serological screening test. To that end, we examined whether elements of MICS were associated with discordant results between these 2 tests. Because previous 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 sensitive to confounding effects of MICS. We also studied demographic, clinical, and laboratory characteristics of MHD patients, including markers of MICS, stratified by results of the 2 mentioned 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[20-23]). 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 malignancy or advanced human immunodeficiency virus disease) were excluded. In the NIED Study cohort, no patient currently 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 measurements were performed, 314 specimens remained for retrospective HCV testing.

Laboratory Evaluation

Predialysis blood samples and postdialysis serum urea nitrogen values were obtained on a midweek day and coincided 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 calculated and used for data analyses in this study.

Serum C-reactive protein and 2 proinflammatory cytokines, 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 turbidometric immunoassay in which a serum sample is mixed with latex beads coated with antihuman C-reactive protein antibodies, forming an insoluble aggregate (WPCI, Osaka, Japan; measured in milligrams per liter; normal range, <3.0 mg/L).[24,25] 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).[26-28] 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).[12] 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.[29-32] 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.[33-35] To evaluate for possible errors in the EIA assay in selected patients, we repeated HCV EIA on the original specimen in all subjects found to be HCV EIA negative (EIA$^-$) and TMA positive (TMA$^+$).

Statistical Methods

Conventional Student *t*-test and analysis of variance were 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, ie, 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
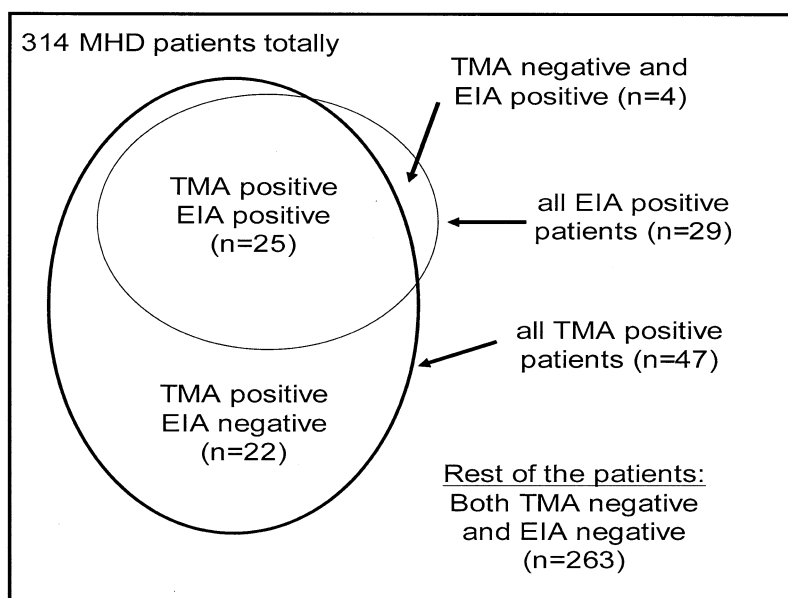
Fig 1.   Diagnostic tests for HCV infection in 314 MHD patients.

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 posi-

tivity 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

Table 1.   Comparison of the TMA and EIA Diagnostic Tests for HCV Infection

	TMA$^+$	TMA$^-$	
EIA$^+$	25 (8)	4 (1)	29 (9)
EIA$^-$	22 (7)	263 (84)	285 (91)
	47 (15)	267 (85)	314 (100)
		Sensitivity (%)	Specificity (%)
EIA$^+$ (TMA as reference)		53	99

NOTE. Values expressed as count (percent) unless noted otherwise.

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

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

NOTE. Count values expressed as percentage, continuous values expressed as mean $\pm$ 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.

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⁺) 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 3. Demographic, Clinical, and Laboratory Characteristics Based on HCV Antibody EIA and HCV RNA by TMA Status

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

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.

lence of HCV 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.[39] However, given that some patients with HCV infection are only intermittently viremic,[40,41] it still is possible that these 4 EIA$^+$/TMA$^-$ patients are truly HCV infected. In a recent study by Sypsa et al,[42] 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

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

Variable (direction and magnitude of change)	Risk of TMA$^+$		Risk of EIA$^+$	
	Odds Ratio (95% CI)	P	Odds Ratio (95% CI)	P
Age (each 10-y decrease)	1.31 (0.99-1.73)	0.06	1.21 (0.84-1.73)	0.3
Female (v male)	1.16 (0.57-2.37)	0.7	1.26 (0.49-3.27)	0.6
Black (v other races)	3.75 (1.76-7.97)	0.001	3.93 (1.43-10.81)	0.008
Diabetes mellitus	2.05 (0.91-4.63)	0.08	0.71 (0.24-2.09)	0.5
Dialysis vintage $\geq$ 12 mo	0.80 (0.37-1.75)	0.6	0.67 (0.24-1.84)	0.4
Body mass index (each 1-k/m^2 decrease)	1.09 (1.02-1.17)	0.01	1.09 (0.99-1.20)	0.07
AST (each 10-U/L increase)	1.45 (1.08-1.94)	0.01	1.51 (1.04-2.18)	0.03
Serum albumin (each 0.1-g/dL decrease)	1.15 (0.99-1.33)	0.06	1.34 (1.10-1.63)	0.004
Serum iron (each 10-ng/mL increase)	1.19 (0.99-1.43)	0.07	1.52 (1.18-1.94)	0.001
Log C-reactive protein (each 1-unit increase)	0.83 (0.50-1.37)	0.5	0.67 (0.34-1.31)	0.24
Log IL-6 (each 1-unit increase)	1.05 (0.68-1.63)	0.8	1.26 (0.74-2.16)	0.4
Log TNF-α (each 1-unit increase)	1.69 (0.91-3.15)	0.10	2.41 (1.06-5.47)	0.04

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.

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,[43,44] but consistent with these subjects having immune defects resulting in a greater frequency of HCV chronicity, such as human immunodeficiency virus–infected subjects.[45-47] In our study, TMA$^+$ MHD 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,[40,41] 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,[6,33,48-51] 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.[6,33,51] We recently found that a serum AST level greater than 25 U/L had an adjusted odds ratio of 4.96 (95% CI, 3.75 to 6.57) for HCV antibody positivity (sensitivity, 50%; specificity, 87%) in a large national database.[5] Similarly, in our study, AST level was significantly greater in TMA$^+$/EIA$^+$ patients compared with the other groups.

In our study, we did not use PCR. PCR assays may be adversely affected by the presence of heparin, a substance commonly used during hemodialysis and often present in blood samples from MHD patients.[12,52,53] Molecular-based assays that detect HCV RNA, such as PCR[12,50,54,55] and TMA,[12,32] are assumed to be the most sensitive diagnostic tests and may pick up additional HCV-infected patients who are EIA$^-$. However, 1 investigation of MHD patients found that TMA appears to be significantly more sensitive than PCR, while maintaining the same high specificity.[12] These findings would be consistent with the observation that TMA is an ultrasensitive HCV RNA qualitative assay that can detect very low amounts of HCV RNA, making it more

sensitive than PCR.[12,29-32,56,57] Several studies showed the superiority and reliability of HCV TMA in detecting HCV infection in the general population compared with HCV PCR.[12,29,56] Krajden et al[56] examined 300 specimens, including 112 samples that were indeterminate by means of EIA, but PCR$^-$, and 79 samples that were EIA$^+$ and PCR$^-$ and showed that TMA was more sensitive than PCR, required less time for test result completion, and had a greater throughput. Comanor et al[29] 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[12] 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.[12,29-32,56,57] 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[58] 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[12] 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.[12,33,50,51] 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.

ACKNOWLEDGMENT

REFERENCES

1. Schneeberger PM, Keur I, van Loon AM, et al: The prevalence and incidence of hepatitis C virus infections among dialysis patients in The Netherlands: A nationwide prospective study. J Infect Dis 182:1291-1299, 2000

2. Sivapalasingam S, Malak SF, Sullivan JF, Lorch J, Sepkowitz KA: High prevalence of hepatitis C infection among patients receiving hemodialysis at an urban dialysis center. Infect Control Hosp Epidemiol 23:319-324, 2002

3. Weinstein T, Tur-Kaspa R, Chagnac A, et al: Hepatitis C infection in dialysis patients in Israel. Isr Med Assoc J 3:174-177, 2001

4. Alavian SM, Einollahi B, Hajarizadeh B, Bakhtiari S, Nafar M, Ahrabi S: Prevalence of hepatitis C virus infection and related risk factors among Iranian haemodialysis patients. Nephrology (Carlton) 8:256-260, 2003

5. Kalantar-Zadeh K, McAllister CJ, Miller LG: Clinical characteristics and mortality-predictability of hepatitis C antibody positivity in hemodialysis patients: A population based study. Nephrol Dial Transplant 2005 (e-pub ahead of print: May 19)

6. Meyers CM, Seeff LB, Stehman-Breen CO, Hoofnagle JH: Hepatitis C and renal disease: An update. Am J Kidney Dis 42:631-657, 2003

7. Saxena AK, Panhotra BR, Sundaram DS, et al: Impact of dedicated space, dialysis equipment, and nursing staff on the transmission of hepatitis C virus in a hemodialysis unit of the Middle East. Am J Infect Control 31:26-33, 2003

8. Espinosa M, Martin-Malo A, Ojeda R, et al: Marked reduction in the prevalence of hepatitis C virus infection in hemodialysis patients: Causes and consequences. Am J Kidney Dis 43:685-689, 2004

9. Nakayama E, Akiba T, Marumo F, Sato C: Prognosis of anti-hepatitis C virus antibody-positive patients on regular hemodialysis therapy. J Am Soc Nephrol 11:1896-1902, 2000

10. Tokars JI, Frank M, Alter MJ, Arduino MJ: National surveillance of dialysis-associated diseases in the United States, 2000. Semin Dial 15:162-171, 2002

11. Rivanera D, Lilli D, Lorino G, et al: Detection of antibodies to hepatitis C virus in dialysis patients. Eur J Epidemiol 9:55-58, 1993

12. Khan N, Aswad S, Shidban H, Aghajani M, Mendez R, Comanor L: Improved detection of HCV infection in hemodialysis patients using a new HCV RNA qualitative assay: Experience of a transplant center. J Clin Virol 30:175-182, 2004

13. Cabana MD, Ensor A, Tunnessen WW Jr: Picture of the month. Half-and-half fingernails. Arch Pediatr Adolesc Med 152:923-924, 1998

14. Carneiro MA, Martins RM, Teles SA, et al: Hepatitis C prevalence and risk factors in hemodialysis patients in central Brazil: A survey by polymerase chain reaction and serological methods. Mem Inst Oswaldo Cruz 96:765-769, 2001

15. Kalantar-Zadeh K, Ikizler TA, Block G, Avram MM, Kopple JD: Malnutrition-inflammation complex syndrome in dialysis patients: Causes and consequences. Am J Kidney Dis 42:864-881, 2003

16. Kalantar-Zadeh K, Supasyndh O, Lehn RS, McAllister CJ, Kopple JD: Normalized protein nitrogen appearance is correlated with hospitalization and mortality in hemodialysis patients with Kt/V greater than 1.20. J Ren Nutr 13:15-25, 2003

17. Woitas RP, Petersen U, Moshage D, et al: HCV-specific cytokine induction in monocytes of patients with different outcomes of hepatitis C. World J Gastroenterol 8:562-566, 2002

18. Chung YM, Park KJ, Choi SY, Hwang SB, Lee SY: Hepatitis C virus core protein potentiates TNF-alpha-induced NF-kappaB activation through TRAF2-IKKbeta-dependent pathway. Biochem Biophys Res Commun 284:15-19, 2001

19. Pawlak K, Pawlak D, Mysliwiec M: Hepatitis intensified oxidative stress, MIP-1beta and RANTES plasma levels in uraemic patients. Cytokine 28:197-204, 2004

20. Colman S, Bross R, Benner D, et al: The Nutritional and Inflammatory Evaluation in Dialysis patients (NIED) Study: Overview of the NIED Study and the role of dietitians. J Ren Nutr 15:231-243, 2005

21. Kalantar-Zadeh K, McAllister CJ, Lehn RS, Lee GH, Nissenson AR, Kopple JD: Effect of malnutrition-inflammation complex syndrome on EPO hyporesponsiveness in maintenance hemodialysis patients. Am J Kidney Dis 42:761-773, 2003

22. Kalantar-Zadeh K, Block G, Humphreys MH, McAllister CJ, Kopple JD: A low, rather than a high, total plasma homocysteine is an indicator of poor outcome in hemodialysis patients. J Am Soc Nephrol 15:442-453, 2004

23. Kalantar-Zadeh K, Kopple JD, Humphreys MH, Block G: Comparing outcome predictability of markers of malnutrition-inflammation complex syndrome in haemodialysis patients. Nephrol Dial Transplant 19:1507-1519, 2004

24. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR: Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 347:1557-1565, 2002

25. Erbagci AB, Tarakcioglu M, Aksoy M, et al: Diagnostic value of CRP and Lp(a) in coronary heart disease. Acta Cardiol 57:197-204, 2002

26. Pecoits-Filho R, Barany P, Lindholm B, Heimburger O, Stenvinkel P: Interleukin-6 is an independent predictor of mortality in patients starting dialysis treatment. Nephrol Dial Transplant 17:1684-1688, 2002

27. Stenvinkel P, Heimburger O, Jogestrand T: Elevated interleukin-6 predicts progressive carotid artery atherosclerosis in dialysis patients: Association with *Chlamydia pneumoniae* seropositivity. Am J Kidney Dis 39:274-282, 2002

28. Beutler B, Cerami A: The biology of cachectin/TNF—A primary mediator of host response. Ann Rev Immunol 7:625-655, 1989

29. Comanor L, Anderson F, Ghany M, et al: Transcription-mediated amplification is more sensitive than conventional PCR-based assays for detecting residual serum HCV RNA at end of treatment. Am J Gastroenterol 96:2968-2972, 2001

30. Gorrin G, Friesenhahn M, Lin P, et al: Performance evaluation of the Versant HCV RNA qualitative assay by using transcription-mediated amplification. J Clin Microbiol 41:310-317, 2003

31. Hendricks DA, Friesenhahn M, Tanimoto L, Goergen B, Dodge D, Comanor L: Multicenter evaluation of the Versant HCV RNA qualitative assay for detection of hepatitis C virus RNA. J Clin Microbiol 41:651-656, 2003

32. Sarrazin C, Teuber G, Kokka R, Rabenau H, Zeuzem S: Detection of residual hepatitis C virus RNA by transcription-mediated amplification in patients with complete virologic response according to polymerase chain reaction-based assays. Hepatology 32:818-823, 2000

33. Saab S, Brezina M, Gitnick G, Martin P, Yee HF Jr: Hepatitis C screening strategies in hemodialysis patients. Am J Kidney Dis 38:91-97, 2001

34. Bonacini M, Lin HJ, Hollinger FB: Effect of coexisting HIV-1 infection on the diagnosis and evaluation of hepatitis C virus. J Acquir Immune Defic Syndr 26:340-344, 2001

35. Chou R, Clark EC, Helfand M: Screening for hepatitis C virus infection: A review of the evidence for the US Preventive Services Task Force. Ann Intern Med 140:465-479, 2004

36. Batty DS Jr, Swanson SJ, Kirk AD, Ko CW, Agodoa LY, Abbott KC: Hepatitis C virus seropositivity at the time of renal transplantation in the United States: Associated factors and patient survival. Am J Transplant 1:179-184, 2001

37. Fabrizi F, Lunghi G, Pagliari B, et al: Molecular epidemiology of hepatitis C virus infection in dialysis patients. Nephron 77:190-196, 1997

38. Castelnovo C, Lunghi G, De Vecchi A, et al: Comparison of three different tests for assessment of hepatitis C virus in dialysis patients. Perit Dial Int 15:241-245, 1995

39. Kamar N, Toupance O, Buchler M, et al: Evidence that clearance of hepatitis C virus RNA after alpha-interferon therapy in dialysis patients is sustained after renal transplantation. J Am Soc Nephrol 14:2092-2098, 2003

40. Umlauft F, Gruenewald K, Weiss G, et al: Patterns of hepatitis C viremia in patients receiving hemodialysis. Am J Gastroenterol 92:73-78, 1997

41. Seelig R, Renz M, Bottner C, Seelig HP: Hepatitis C virus infections in dialysis units: Prevalence of HCV-RNA and antibodies to HCV. Ann Med 26:45-52, 1994

42. Sypsa V, Psichogiou M, Katsoulidou A, et al: Incidence and patterns of hepatitis C virus seroconversion in a cohort of hemodialysis patients. Am J Kidney Dis 45:334-343, 2005

43. al-Mugeiren M, al-Rasheed S, al-Salloum A, et al: Hepatitis C virus infection in two groups of paediatric patients: One maintained on haemodialysis and the other on continuous ambulatory peritoneal dialysis. Ann Trop Paediatr 16:335-339, 1996

44. Chanpong GF, Laras K, Sulaiman HA, et al: Hepatitis C among child transfusion and adult renal dialysis patients in Indonesia. Am J Trop Med Hyg 66:317-320, 2002

45. Yoo TW, Donfield S, Lail A, Lynn HS, Daar ES: Effect of hepatitis C virus (HCV) genotype on HCV and HIV-1 disease. J Infect Dis 191:4-10, 2005

46. Daar ES, Lynn H, Donfield S, et al: Relation between HIV-1 and hepatitis C viral load in patients with hemophilia. J Acquir Immune Defic Syndr 26:466-472, 2001

47. Daar ES, Lynn H, Donfield S, et al: Hepatitis C virus load is associated with human immunodeficiency virus type 1 disease progression in hemophiliacs. J Infect Dis 183:589-595, 2001

48. Fabrizi F, Poordad FF, Martin P: Hepatitis C infection and the patient with end-stage renal disease. Hepatology 36:3-10, 2002

49. Natov SN, Pereira BJ: Routine serologic testing for hepatitis C virus infection should be instituted among dialysis patients. Semin Dial 13:393-398, 2000

50. Pol S, Romeo R, Zins B, et al: Hepatitis C virus RNA in anti-HCV positive hemodialyzed patients: Significance and therapeutic implications. Kidney Int 44:1097-1100, 1993

51. Saab S, Martin P, Brezina M, Gitnick G, Yee HF Jr: Serum alanine aminotransferase in hepatitis C screening of patients on hemodialysis. Am J Kidney Dis 37:308-315, 2001

52. Zacks SL, Fried MW: Hepatitis B and C and renal failure. Infect Dis Clin North Am 15:877-899, 2001

53. Noiri E, Nakao A, Oya A, Fujita T, Kimura S: Hepatitis C virus in blood and dialysate in hemodialysis. Am J Kidney Dis 37:38-42, 2001

54. Chan TM, Lok AS, Cheng IK, Chan RT: Prevalence of hepatitis C virus infection in hemodialysis patients: A longitudinal study comparing the results of RNA and antibody assays. Hepatology 17:5-8, 1993

55. Alter MJ, Kuhnert WL, Finelli L: Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. MMWR Morb Mortal Wkly Rep 52:1-13, 15; quiz, CE11-CE14, 2003

56. Krajden M, Ziermann R, Khan A, et al: Qualitative detection of hepatitis C virus RNA: Comparison of analytical sensitivity, clinical performance, and workflow of the Cobas Amplicor HCV test version 2.0 and the HCV RNA transcription-mediated amplification qualitative assay. J Clin Microbiol 40:2903-2907, 2002

57. Comanor L, Elkin C, Leung K, et al: Successful HCV genotyping of previously failed and low viral load specimens using an HCV RNA qualitative assay based on transcription-mediated amplification in conjunction with the line probe assay. J Clin Virol 28:14-26, 2003

58. Fong T, Valinluck B, Gozindarajan S, Charoneau F, Adkins B, Redeker A: Short-term prednisone therapy affects aminotransferase activity and hepatitis C RNA levels in chronic hepatitis C. Gastroenterology 107:196-199, 1994