JCM Journals.ASM.org

Impact of Isolated Hepatitis C Virus (HCV) Core-Specific Antibody Detection and Viral RNA Amplification among HCV-Seronegative Dialysis Patients at Risk for Infection

Guillermina Barril,[a] Juan A. Quiroga,[b] María Dolores Arenas,[c] Mario Espinosa,[d] Nuria García-Fernández,[e] Secundino Cigarrán,[f] José A. Herrero,[g] Gloria del Peso,[h] Pilar Caro,[i] Rebeca García-Agudo,[j] Yésica Amézquita,[k] Ana Blanco,[l] Pilar Martínez-Rubio,[m] José M. Alcázar,[n] Emilio González-Parra,[o]* Adoración Martín-Gómez,[p] Inmaculada Castillo,[b] Javier Bartolomé,[b] Vicente Carreño[b]

Nephrology, Hospital Universitario de La Princesa, Madrid, Spain[a]; Fundación Estudio Hepatitis Virales, Madrid, Spain[b]; Nephrology, Hospital Perpetuo Socorro, Alicante, Spain[c]; Nephrology, Hospital Reina Sofía, Córdoba, Spain[d]; Nephrology, Clínica Universitaria de Navarra, Pamplona, Spain[e]; Nephrology, Hospital da Costa, Burela, Lugo, Spain[f]; Nephrology, Hospital Clínico San Carlos, Madrid, Spain[g]; Nephrology, Hospital La Paz, Madrid, Spain[h]; Nephrology, Clínica Rúber, Madrid, Spain[i]; Complejo Hospitalario La Mancha-Centro, Alcázar de San Juan, Ciudad Real, Spain[j]; Clínica Fuensanta, Madrid, Spain[k]; Dial Centro, Madrid, Spain[l]; ICN El Pilar, Madrid, Spain[m]; Nephrology, Hospital 12 de Octubre, Madrid, Spain[n]; Nephrology, Hospital Central de la Defensa, Madrid, Spain[o]; Nephrology, Hospital de Poniente, Almería, Spain[p]

Amplification of hepatitis C virus (HCV) RNA from blood detected occult HCV infections in 30.9% of 210 HCV-seronegative dialysis patients with abnormal liver enzyme levels that had evaded standard HCV testing practices. Isolated HCV core-specific antibody detection identified three additional anti-HCV screening-negative patients lacking HCV RNA amplification in blood who were considered potentially infectious. Together, these findings may affect management of the dialysis setting.

Hepatitis C virus (HCV) infection is prevalent among patients with end-stage renal disease (ESRD) who are undergoing maintenance dialysis, and it has detrimental effects on disease progression and patient survival times, although HCV-related liver disease is mostly mild and asymptomatic (1–3). Accurate HCV diagnosis is limited by the lack of detectable anti-HCV antibodies in some viremic patients (4). Also, HCV RNA has been detected in blood cells from HCV-seronegative (negative for anti-HCV and serum HCV RNA) hemodialysis (HD) patients with abnormal alanine aminotransferase (ALT) and/or gamma-glutamyl transpeptidase (GGTP) values (i.e., occult HCV infection) (5). Such patients can be infectious and contribute to HCV spread within dialysis units but remain undiagnosed with standard diagnostic techniques (6). This study aimed to identify occult HCV infections among HCV-seronegative dialysis patients with abnormal liver enzyme levels that had evaded standard HCV testing practices.

(This work was presented in part as a poster at the 64th Annual Meeting of the American Association for the Study of Liver Diseases, Washington, DC, 1 to 5 November 2013 [7].)

Fifteen Spanish dialysis units enrolled patients for substitutive hemodialysis or peritoneal dialysis (PD) therapy. The inclusion criteria were (i) ALT levels above 28 IU/liter (8) and/or abnormal GGTP levels (>43 IU/liter) for more than 6 months before study entry; (ii) negative results for markers of HCV (anti-HCV and HCV RNA), HIV (anti-HIV), and hepatitis B virus (HBV) (HBV surface antigen and DNA); and (iii) exclusion of other causes of liver disease. Each participating center used a licensed anti-HCV screening assay (most centers used one of the following: Architect anti-HCV or IMX HCV [Abbott Laboratories, Chicago, IL], Elecsys anti-HCV [Roche Diagnostics, Rotkreuz, Switzerland], Advia Centaur HCV [Siemens Healthcare Diagnostics, Erlangen, Germany], or Vitros anti-HCV [Ortho-Clinical Diagnostics, Buckinghamshire, United Kingdom]). Anti-HCV antibodies (Innotest HCV Ab IV; Innogenetics, Ghent, Belgium) and HCV RNA were retested centrally at a single center, which confirmed that 210 subjects (134 male and 76 female subjects; median age, 69 years [range, 25 to 87 years]) fulfilled the inclusion criteria. The median duration of dialysis was 36 months (range, 6 to 264 months); the median ALT and GGTP levels prior to study entry were 26 IU/liter (range, 5 to 180 IU/liter) and 69 IU/liter (range, 10 to 160 IU/liter), respectively. The etiology of kidney disease was diabetes mellitus in 40 cases (19.1%), glomerulonephritis in 27 (12.9%), high blood pressure in 21 (10.0%), interstitial nephropathy in 16 (7.6%), and polycystic kidney in 11 (5.2%), whereas 95 patients (45.2%) suffered from other nephropathies. This study was approved by the coordinating center's ethics committee and was conducted according to the Helsinki Declaration; written consent was obtained from all patients. Serum and peripheral blood mononuclear cell (PBMC) samples were collected at study entry. The HCV RNA 5′ noncoding region in total RNA extracted from PBMCs and ultracentrifuged serum (2 ml) was amplified by quantitative real-time PCR, as reported previously (9, 10). Each test run included repeatedly HCV RNA-negative serum or PBMC samples from healthy volunteers and additional negative controls. A standard curve was constructed with 10-fold dilutions of synthetic genomic HCV RNA and was used for quantification of HCV RNA in PBMCs and serum (assay sensitivity of 3 HCV RNA copies per reaction, as reported elsewhere [9, 10]). The specificity of HCV RNA amplification was demonstrated by phylogenetic analysis of

Received 8 May 2014 Returned for modification 8 May 2014
Accepted 13 May 2014
Published ahead of print 21 May 2014
Editor: Y.-W. Tang
Address correspondence to Vicente Carreño, fehvhpa@fehv.org.
* Present address: Emilio González-Parra, Nephrology, Fundación Jiménez Díaz, Madrid, Spain.
G.B. and J.A.Q. contributed equally to this work.

doi:10.1128/JCM.01339-14

HCV core sequences amplified from ultracentrifuged serum and HCV RNA-positive PBMCs from HD patients (5, 10).

HCV core-specific antibodies were detected using an immunoassay with enhanced sensitivity (anti-HCV core high-sensitivity enzyme-linked immunosorbent assay [ELISA] kit; Diater Laboratories, Madrid, Spain) that uses a conserved HCV core region based on an investigational anti-HCV core immunoassay (11). Testing was performed with prediluted (1:10) samples according to the supplier's instructions; sample-to-cutoff absorbance ratios (absorbance index [AI] values) of ≥1.2 were considered to indicate reactivity. The assay has shown a diagnostic sensitivity of 100% for chronic hepatitis C genotypes 1 to 6 and specificities of 100% and 99.7% among blood donor samples and clinical specimens, respectively (12). Anti-HCV core-positive samples were confirmed by a peptide inhibition assay, as reported previously (11), and antigenic reactivity was characterized by a supplemental immunoblot assay (Inno-LIA HCV Score [Innogenetics], with reported sensitivity and specificity of >80% and >92%, respectively, among low-titer antibody samples, blood donations, or seroconversion panels). Categorical variables were compared using the chi-square test (or Fisher's exact test, when applicable). Continuous variables were compared using the nonparametric Mann-Whitney *U* test. All reported *P* values are two-tailed.

Overall, 65/210 anti-HCV-negative dialysis patients (30.9%) were classified as having occult HCV infections (HCV RNA detectable in PBMC and/or ultracentrifuged serum samples). Patients with occult HCV infections were younger than those without detectable HCV RNA (median age, 63 years [range, 30 to 86 years] versus 71 years [range, 25 to 87 years], respectively; $P < 0.005$) and had greater ALT levels prior to study entry (median level, 27 IU/liter [range, 6 to 144 IU/liter] versus 19 IU/liter [range, 6 to 90 IU/liter], respectively; $P < 0.001$). No differences regarding the mode and duration of dialysis treatment, etiology of kidney disease, gender, blood transfusion, or GGTP levels were found (data not shown).

Anti-HCV core antibodies were detected for 11/210 dialysis patients (5.2%), including 4/65 (6.2%) positive for HCV RNA (in PBMC and/or ultracentrifuged serum samples) and 7/145 (4.8%) without detectable HCV RNA. Table 1 summarizes the characteristics of these 11 patients. HCV core-specific antibody reactivity was inhibited ≥50% in four cases. Thus, the anti-HCV core assay detected 1.5% of HCV-seronegative dialysis patients (1/65 patients) with occult HCV infections and 2.1% of HCV RNA-negative patients (3/145 patients; no occult infection) (specificity, 97.9%). Most patients had been screened with sensitive anti-HCV chemiluminescent immunoassays (Table 1). One confirmed anti-HCV core-positive sample reacted weakly with the C1 (core) band, leading to an indeterminate test result with the supplemental anti-HCV immunoblot assay (patient 5 in Table 1); faint reactivity with the C1 and/or C2 bands was noted for other samples, although the samples were scored negative. Overall, sera from 3/4 patients with confirmed anti-HCV core reactivity showed faint-to-weak core antigen-specific bands. When the threshold for detection was set at an AI of 1.0 (below the AI level of 1.2 recommended by the supplier), confirmed anti-HCV core reactivity increased to 3.8% (8/210 patients), including 3/65 patients (4.6%) with and 5/145 patients (3.4%) without virological markers of occult HCV infection (Table 2).

In this study, we found that 30.9% of ESRD patients undergoing dialysis (either HD or PD) had occult HCV infections (HCV

TABLE 1 Characteristics of patients with positive results in the anti-HCV core high-sensitivity ELISA

Patient no.	Age/sex[a]	Dialysis mode/ duration (mo)	ALT level (IU/liter)	GGTP level (IU/liter)	HCV RNA in PBMCs (copies/μg RNA)	HCV RNA in serum (copies/ml)	Occult HCV infection	Anti-HCV assay (Innotest) result	Anti-HCV core assay (Diater) result	AI	Inhibition (%)	Antigen reactivity in supplemental anti-HCV assay (Inno-LIA)	Anti-HCV screening test
1	70/F	HD/12	66	20	0	0	No	Negative	Positive	1.36	23	Negative	CMIA
2	71/M	HD/56	28	30	1.3×10^4	0	Yes	Negative	Positive	1.32	<5	Negative (faint C1 and C2)	MEIA
3	74/M	HD/36	28	35	0	8.0×10^1	Yes	Negative	Positive	1.34	<5	Negative (faint C1)	CMIA
4	52/F	HD/9	19	118	1.3×10^5	0	Yes	Negative	Positive	2.20	>99	Negative (faint C1)	ECLIA/CMIA
5	69/M	PD/42	12	70	0	0	No	Negative	Positive	1.99	85	Indeterminate (1+ C1)	CIA/CMIA
6	35/M	HD/12	34	69	0	0	No	Negative	Positive	1.28	<5	Negative	CMIA
7	48/M	HD/11	6	52	0	0	No	Negative	Positive	1.60	61	Negative (faint C2)	CMIA
8	82/F	HD/20	20	75	0	0	No	Negative	Positive	1.28	59	Negative	CMIA
9	72/F	HD/38	29	660	3.2×10^4	0	Yes	Negative	Positive	1.43	32	Negative (faint NS4)	CMIA
10	67/M	HD/163	18	59	0	0	No	Negative	Positive	1.46	7	Negative (faint C1 and C2)	CMIA
11	67/M	HD/36	31	72	0	0	No	Negative	Positive	2.35	5	Negative	ECLIA

[a] F, female; M, male; HD, hemodialysis; PD, peritoneal dialysis; AI, absorbance index; CMIA, chemiluminescent microparticle immunoassay (Architect; Abbott Laboratories); MEIA, microparticle enzyme immunoassay (IMX; Abbott Laboratories); ECLIA, electrochemiluminescent immunoassay (Elecsys; Roche Diagnostics); CIA, chemiluminescent immunoassay (Vitros; Ortho Diagnostics).

TABLE 2 Comparison of reactivity in the anti-HCV core high-sensitivity ELISA among dialysis patients, using different values for the threshold for positivity

Test parameter	No. of anti-HCV core-positive cases with AI of:				
	≥1.2	≥1.1	≥1.0	≥0.9	≥0.75[a]
No. with positive HCV RNA status (PBMCs and/or ultracentrifuged serum) (no. confirmed)[b]	4 (1)	6 (3)	6 (3)	6 (3)	13 (NA)
No. with negative HCV RNA status (PBMCs and ultracentrifuged serum) (no. confirmed)[b]	7 (3)	8 (3)	12 (5)	18 (6)	36 (NA)
Total no. positive (no. confirmed)[b] [% positivity for total/confirmed cases]	11 (4) [5.2/1.9]	14 (6) [6.7/2.9]	18 (8) [8.6/3.8]	24 (9) [11.4/4.3]	49 (NA) [23.3/NA]
Increased detection (%) in dialysis patients with occult HCV infections ($n = 65$)[c]	1.5	4.6	4.6	4.6	NA
Specificity (%) in dialysis patients negative for markers of occult HCV infection ($n = 145$)[c]	97.9	97.9	96.6	95.9	NA

[a] An AI of 0.75 corresponds to the cutoff value for the investigational assay reported in reference 11. NA, not available.
[b] Numbers in parentheses refer to anti-HCV core-positive cases confirmed by the inhibition test.
[c] Values calculated considering only confirmed anti-HCV core-positive individuals.

RNA detected in PBMC samples and/or in serum samples after ultracentrifugation to concentrate viral particles), in contrast to the 45% reported previously (5). It is likely that the prevalence of occult HCV infections is decreasing due to more-efficient diagnostic protocols and transmission-prevention measures (1, 3, 13, 14); however, HCV affects kidney function, and mortality rates are higher among HD patients with occult HCV infections (5). We also observed HCV core-specific antibody responses among patients with anti-HCV-negative results in licensed screening tests. Reactivity was confirmed by a peptide inhibition assay in four cases; unconfirmed anti-HCV core reactivity might represent false-positive results, although HCV RNA could be amplified in PBMC or serum samples from some individuals. This suggested that relatively low-avidity anti-HCV core antibodies were present but could be only partially inhibited; indeed, HCV-specific antibody avidity may be altered in dialysis patients (15). Also, most sera showed faint-to-weak HCV core-specific reactions in supplemental anti-HCV immunoassays, as reported for occult HCV infections without renal impairment (11). Although the overall value of the anti-HCV core test appears relatively low, a major impact of this work is the identification of a subset of HCV-seronegative dialysis patients who evade current diagnostic protocols but must be considered potentially infectious, even lacking HCV RNA amplification in blood (3, 8).

False-negative anti-HCV results occur frequently among dialysis patients (14), possibly due to immune dysfunction (4). The threshold to distinguish between anti-HCV core-positive and anti-HCV core-negative results might be lowered in order to define acceptable risks of missing HCV infections among dialysis patients. This modification would help to increase overall sensitivity, without compromising test specificity, in assessing the risk of occult HCV infection in blood, although further studies are needed. Occult infections may affect viral reactivation and disease progression, the risk of HCV transmission within dialysis units, and intrafamilial spread (5, 8). Patients with false-negative results would not be appropriately counseled while undergoing dialysis or after kidney transplantation (2, 3). In conclusion, we recommend monitoring dialysis patients and testing them at regular intervals to detect any HCV-specific antibody reactivity and/or viral burden, so that the most appropriate management can be adopted (3).

ACKNOWLEDGMENTS

This work was supported in part by research grants from the Fundación Mutua Madrileña (Madrid, Spain), the Fundación de Investigaciones Biomédicas (Madrid, Spain), and Diater Laboratories (Madrid, Spain).

We thank Miguel A. Ruiz from Diater for technical support.

We declare no conflicts of interest.

REFERENCES

1. **Jadoul M, Barril G.** 2012. Hepatitis C in hemodialysis: epidemiology and prevention of hepatitis C virus transmission. Contrib. Nephrol. **176:**35–41. http://dx.doi.org/10.1159/000333761.
2. **Fabrizi F, Dixit V, Messa P, Martin P.** 2012. Hepatitis C-related liver disease in dialysis patients. Contrib. Nephrol. **176:**42–53. http://dx.doi.org/10.1159/000332379.
3. **Kidney Disease: Improving Global Outcomes (KDIGO).** 2008. KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. Kidney Int. **73**(Suppl 109):S1–S99. http://dx.doi.org/10.1038/ki.2008.81.
4. **Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases.** 2009. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology **49:**1335–1374. http://dx.doi.org/10.1002/hep.22759.
5. **Barril G, Castillo I, Arenas MD, Espinosa M, Garcia-Valdecasas J, Garcia-Fernández N, González-Parra E, Alcazar JM, Sánchez C, Diez-Baylón JC, Martinez P, Bartolomé J, Carreño V.** 2008. Occult hepatitis C virus infection among hemodialysis patients. J. Am. Soc. Nephrol. **19:**2288–2292. http://dx.doi.org/10.1681/ASN.2008030293.
6. **Castillo I, Pardo M, Bartolomé J, Ortiz-Movilla N, Rodríguez-Iñigo E, de Lucas S, Salas C, Jiménez-Hefferman JA, Pérez-Mota A, Graus J, López-Alcorocho JM, Carreño V.** 2004. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. J. Infect. Dis. **189:**7–14. http://dx.doi.org/10.1086/380202.
7. **Quiroga JA, Barril G, Arenas D, Espinosa M, GarciaFernandez N, Cigarran S, Herrero J, del Peso G, Caro P, Garcia R, Amezquita Y, Blanco A, Martinez P, Alcazar JM, Gonzalez-Parra E, Dfaz-Bailon JC, Martin A, Castillo I, Bartolomé J, Carreño V.** 2013. Evaluation of the anti-HCV Core High Sensitivity® ELISA test among anti-HCV screening-negative dialysis patients at risk of occult HCV infection. Hepatology **58**(Suppl 1):912A.
8. **Barril G, González Parra E, Alcázar R, Arenas D, Campistol JM, Caramelo C, Carrasco M, Carreño V, Espinosa M, García Valdecasas J, Górriz JL, López MD, Martín L, Ruiz P, Terruel JL, Spanish Society of Nephrology.** 2004. Guidelines on hemodialysis-associated viral infections. Nefrologia **24**(Suppl 2):43–66. (In Spanish.)

9. **Castillo I, Bartolomé J, Quiroga JA, Barril G, Carreño V.** 2010. Diagnosis of occult hepatitis C without the need for a liver biopsy. J. Med. Virol. **82:**1554–1559. http://dx.doi.org/10.1002/jmv.21866.
10. **Bartolomé J, López-Alcorocho JM, Castillo I, Rodríguez-Iñigo E, Quiroga JA, Palacios R, Carreño V.** 2007. Ultracentrifugation of serum samples allows detection of hepatitis C virus RNA in patients with occult hepatitis C. J. Virol. **81:**7710–7715. http://dx.doi.org/10.1128/JVI.02750-06.
11. **Quiroga JA, Castillo I, Llorente S, Bartolomé J, Barril G, Carreño V.** 2009. Identification of serologically silent occult hepatitis C virus infection by detecting immunoglobulin G antibody to a dominant HCV core peptide epitope. J. Hepatol. **50:**256–263. http://dx.doi.org/10.1016/j.jhep.2008.08.021.
12. **Quiroga JA, Alcover J, Bartolomé J, Castillo I, de Lecea C, Palacios R, Carreño V.** 2012. A new high sensitivity anti-HCV Core kit allows detecting hepatitis C virus infected individuals not detected by other available anti-HCV assays. Hepatology **56**(Suppl 1)**:**672A–673A.
13. **Centers for Disease Control and Prevention.** 2001. Recommendations for preventing transmission of infections among chronic hemodialysis patients. MMWR Recomm. Rep. **50**(RR05)**:**1–43.
14. **Kamili S, Drobeniuc J, Araujo AC, Hayden TM.** 2012. Laboratory diagnostics for hepatitis C virus infection. Clin. Infect. Dis. **55**(Suppl 1)**:** S43–S48. http://dx.doi.org/10.1093/cid/cis368.
15. **Ward KN, Dhaliwal W, Ashworth KL, Clutterbuck EJ, Teo CG.** 1994. Measurement of antibody avidity for hepatitis C virus distinguishes primary antibody responses from passively acquired antibody. J. Med. Virol. **43:**367–372. http://dx.doi.org/10.1002/jmv.1890430409.