High Prevalence of Hepatitis C Virus Infection in Hemodialysis Patients from One Dialysis Unit in Slovenia

K.		Seme ^a
M.		Poljak ^a
S.		Žužek-Rešek ^b
T.		Avšič-Županc ^a

^aInstitute of Microbiology, Medical Faculty of Ljubljana, and ^bHemodialysis Unit Izola, Medical Center Koper, Izola, Slovenia

K. Seme, MD, Institute of Microbiology, Medical Faculty of Ljubljana, SLO-61 105 Ljubljana (Slovenia)

Dear Sir,

Hemodialysis patients belong to a high-risk group for exposure to hepatitis C virus (HCV). The reported prevalence of HCV antibodies in hemodialysis patients varies from 1 to 71% [1-3]. However, antibody assays can only provide evidence of exposure to HCV, but they are unable to differentiate between previous and ongoing infections or otherwise between infectious and noninfectious patients. The polymerase chain reaction (PCR), which allows detection of viral genome sequences in biological specimens, is at present the only method available to demonstrate viremia in patients with HCV infection [4]. Recent studies on the prevalence of HCV viremia in hemodialysis patients indicate that a high proportion (66-95%) of anti-HCV-positive patients and a variable proportion (2.5-17%) of seronegative patients are viremic [3, 5, 6].

In the present study, we applied serological assays and reverse-transcription PCR to evaluate the HCV infection in 47 patients with end-stage renal disease treated with hemodialysis in one dialysis unit in Slovenia.

Serum samples were tested for HCV antibodies using the second-generation enzyme-linked immunosorbent assay (UBI HCV EIA, Organon Teknika). The assay is based on synthetic peptides which correspond to the structural (core) and nonstructural portions (NS3 and NS4) of HCV. Anti-HCV immunoreactivity determined by EIA was confirmed by a second-generation immunoblot assay (RIBAII, Chiron Corp. and Ortho Diagnostic Systems), which uses four recombinant HCV antigens (5-1-1, c100-3, c33c and c22-3) fused to human superoxide dismutase, and by a third-generation immunoblot assay (LiaTek HCV III, Organon Teknika), which uses recombinant and synthetic peptides representing the following HCV antigens:

core, E2/NS1, NS3, NS4 and NS5.

HCV viremia was detected in the same serum samples by a commercially available PCR assay, Amplicor Hepatitis C Virus Test, recently developed by Roche Molecular Systems. The test is based on four major processes: reverse transcription of viral RNA to cDNA, PCR amplification using biotinylated primers, hybridization of the amplified product to a specific nucleic acid probe captured to the microtiter plate and detection of the amplified product by the biotin-avidin-horseradish-peroxidase assay. The primers and the probe used in the

Amplicor HCV test are selected from the highly conserved 5' terminus of the HCV genome. The assay includes a uracil-N-glycosylase sterilization system to prevent false-positive results due to PCR carryover. All serum samples were tested in duplicate and in parallel with our representative positive and negative controls.

Serum samples of 16/47 (34%) patients were anti-HCV reactive as determined by EIA. Eleven out of 16 patients were confirmed by the second-generation immunoblot assay to be anti-HCV positive. The remaining 5 patients were indeterminate. The third-generation immunoblot assay confirmed 14 EIA-reactive patients as anti-HCV positive, and 2 patients were indeterminate.

All of the anti-HCV-positive patients determined by the third-generation immunoblot assay were HCV RNA positive by the Amplicor HCV test and the 2 anti-HCV-indeterminate patients were HCV RNA negative. All anti-HCV-positive patients and 3 anti-HCV-indeterminate patients deter-

M 1 2 3 4 5 6 7

12 3 4 5 6 7

Fig. 1. Detection of HCV RNA in serum samples by the Amplicor HCV test. Agarose gel analyses of HCV-specific PCR amplification products (244 bp; top) and confirmation of specificity of PCR products by the biotin-avidin-horseradish-peroxidase assay (bottom). Lane M = DNA ladder, 123 bp (Gibco BRL); lanes 1, 3, 5 and 7 = HCV-RNA-negative serum samples of anti-HCV-negative patients; lanes 2 and 4 = HCV-RNA-positive serum samples of anti-HCV-positive patients; lane 6 = HCV-RNA-positive serum sample of an anti-HCV-negative patient.

mined by the second-generation immunoblot assay were viremic. One out of 31 (3.2%) anti-HCV-negative patients had detectable levels of HCV RNA in his serum (fig. 1). Alanine aminotransferase levels carry no predictive value of HCV infection,

because they proved to be repeatedly normal in 12 of 15 (80%) viremic patients.

In this study, we demonstrated that the third-generation immunoblot assay was more useful than the second-generation immunoblot assay in detecting HCV infection. We got 5 indeterminate results by the second-generation test, while the third-generation immunoblot assay provided a clear confirmation of seropositivity in 3 of these cases and left only 2 indeterminate. Our results show that a high proportion (31.9%) of he-modialysis patients from one small dialysis unit in Slovenia have HCV viremia, the vast majority of them being anti-HCV positive. We also found 1 patient from this unit with seronegative HCV infection. Anti-HCV positivity, as detected by the third-generation immunoblot assay, was the best predictor of HCV viremia in our study. Despite a good correlation found between circulating anti-HCV and HCV RNA in serum, we agree with others that the active HCV infection cannot be predicted by serology and that PCR is the only method which enables the discrimination between ongoing and previous HCV infection and thus makes it possible to determine potential infectivity of patients [3, 5, 6]. We found the Amplicor HCV test a rapid, simple and useful PCR assay for the detection of HCV RNA in serum samples.

References

Mortimer PP, Coher BJ, Litton PA, Vander-velde EM, Bassedine MF, Brind AM, Hambling MH: Hepatitis C virus antibody. *Lancet* 1989;ii:798.

Kapoor M, El-Reshaid K, Al-Mufti S, Sanad NA, Koshy A: Is dialysis environment more important than blood transfusion in transmission of hepatitis C virus during hemodialysis? *Vox Sang* 1993;65:331.

Silini E, Bono F, Cerino A, Piazza V, Solcia E, Mondelli MU: Virological features of hepatitis C virus infection in hemodialysis patients. *J Clin Microbiol* 1993;31:2913-2917.

Alter MJ: The detection, transmission and outcome of hepatitis C virus infection. *Infect Agents Dis* 1993;2:155-166.

Sheu JC, Lee SH, Wang JT, Shih LN, Wang TH, Chen DS: Prevalence of anti-HCV and HCV viremia in hemodialysis patients in Taiwan. *J Med Virol* 1992;37:108-112.

Bukh J, Wantzin P, Krogsgaard K, Knudsen F, Purcell RH, Miller RH: High prevalence of hepatitis C virus (HCV) RNA in dialysis patients: Failure of commercially available antibody tests to identify a significant number of patients with HCV infection. *J Infect Dis* 1993; 168:1343-1348.

100

Seme/Poljak/Zuzek-Resek/Avsic-Zupanc
Hepatitis C Virus Infection in Slovenia