Detection of Hepatitis C Virus in Dialysate and in Blood Ultrafiltrate of HCV-Positive Patients

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Dear Sir,

Hepatitis C virus (HCV) is a major cause of hepatitis in haemodialysis patients. The prevalence of anti-HCV antibodies increases significantly with increasing age and frequency of haemodialysis. The analysis of known risk factors such as blood transfusions, status of hepatitis B and HIV infection, and intravenous drug addiction appears to leave an unaccounted residual risk which is directly related to the duration of exposure to dialytic treatment [1-3]. Therefore other, as yet undefined, parenteral routes of transmission of HCV may exist in the haemodialysis setting.

With the aim of investigating unrecognized possible routes of HCV transmission in dialysis patients we examined samples of fluid collected at the dialysate outlet of filters and samples of blood ultrafiltrate during the dialytic treatment of HCV-positive patients with the polymerase chain reaction (PCR) for the presence of HCV RNA.

Four 100-ml samples were collected at the end of sessions (three dialysates from poly-methylmetacrylate, cuprophane and polysulphone membranes, respectively, and one ultrafiltrate from polysulphone). All samples were immediately processed by centrifugation through Centriprep 100 (Amicon Division, Beverly, Mass., USA) cartridges to obtain a 100-fold concentration. RNA extraction was performed with the acid guanidinium thiocyanate-phenol-chloroform method [4] with the addition of 1 µg glycogen as a carrier prior to isopropanol precipitation. Complementary DNA (cDNA) synthesis (Promega Corporation, Madison, Wisc., USA) was primed from

the core region of HCV RNA. Nested PCR was performed using primer sequences located in 5' untranslated region (235 bp product, nt 275-40) [5] and in the core region (143 bp product, nt 148-291) [6] of the viral genome. Strict precautions to prevent contamination were adopted; negative controls were introduced during RNA extraction and processed throughout cDNA synthesis and PCR.

In two samples, one dialysate and one ultrafiltrate, both from polysulphone membranes, we obtained PCR products of the appropriate size visualized on ethidium bromide-stained

polyacrylamide gels. In both cases we found positivity for the 5' untranslated region as well as for a core HCV type II sequence, consistent with the viral subtype previously identified in the patients.

The passage of viral particles across dialysis membranes is likely to occur through microruptures of capillaries giving rise to leakages of blood, below the sensitivity of the blood leak detector, into the dialysis fluid. They are likely to happen as the result of intradialytic mechanical stress of membranes and/or microclotting of some hollow fibres followed by their obstruction in a system with high blood flow and pressure. This event could be influenced by many factors, such as the type and preparation of membrane, type and duration of dialysis, transmembrane pressures, incorrect use of heparin and titre of viraemia in the dialysis patient, all of which will require further evaluation.

Our findings indicate the existence of a potential risk of cross-infection of HCV by dialysis fluids in patients utilizing high flux membranes with backfiltration of dialysis fluid into the blood. This would suggest the utility to adopt precautions such as the segregation of machines used for HCV-positive patients and disinfection of machines after each dialytic session.

References

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