Is Hepatitis C Virus RNA Detectable in Dialysis Ultrafiltrate?

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ised [4]. This problem is particularly troublesome since the majority of the dialysis centers do not have enough devices to isolate anti-HCV-positive patients, and, getting to the root of the problem, it is not clear whether this procedure must be considered absolutely necessary or not. We investigated this problem recently (presented at the 1994 Meeting of the American Society of Nephrology). We studied HCV-RNA in hemodialysis ultrafiltrates from HCV-positive patients. We considered 3 anti-HCV-Ab-positive hemodialyzed patients (Ortho 2, a second-generation ELISA). Two of them were HCV-RNA positive. HCV-RNA was tested in blood, in ultrafiltrate samples, and on the dialysis membrane surface during two hemodialysis sessions in these subjects. Blood and ultrafiltrates were collected at the beginning, after 30, 60 and 120 min and at the end of the session. Ultra-

Table 1. Relations among reference chimp serum dilutions and viremia expressed as CID₅₀/ml, g Eq/ml or RT-PCR score

Chimp serum dilutions

CID₅₀/ml

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Dear Sir,

Hepatitis C virus (HCV) infection is a challenging problem in every dialysis unit. The prevalence of patients on regular hemodialysis treatment, who are positive for anti-HCV antibodies (Abs), is much higher than in the corresponding general population, ranging in Europe from 1.6 to 28.6% [1]. Blood transfusions, particularly from anti-HCV Ab-untreated donors, may be an important form of infection transmission, and the improvement in uremic anemia with recombinant human erythropoietin treatment is expected to decrease the incidence of new HCV infections. However, each dialysis center has experienced several cases of new HCV infections in patients who had not received transfusions of whole blood or its derivatives and lack other risk factors, including hepatitis B and HIV infections or intravenous drug administration. Over the same period it became evident that HCV infection in uremic patients is much more harmful than

considered before and may be life threatening particularly after kidney transplantation and prolonged immunosuppression [2].

For these reasons, several attempts have recently been made to investigate the other forms of infection which may be responsible for HCV diffusion in hemodialyzed patients. Since the prevalence of HCV patients increases with dialytic age [3], the possibility that the virus passes through the dialysis membranes in the ultrafiltrate, thus making the dialysis monitor an instrument in HCV infection transmission, has recently been

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filtrates collected at the end of dialysis were concentrated 100 times. At the end of hemodialysis the dialysis membranes (two flat-plate dialyzers with cuprophan and two AN 69 membranes) were collected and stored in PBS at -80 °C until processed.

g Eq/ml

RT-PCR

score

HCV-RNA was measured by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted from 100 µl of serum or ultrafiltrate samples and from 1 cm² of dialysis membranes incubated in 100 µl 0.05 M trypsin, EDTA 0.02 M in 0.15 M NaCl according to the method of Chomczynsky and Sacchi [5, 6]. After a reverse transcription step, cDNA was amplified using a primer of the 5' non-coding region of the HCV genome [7] in a single-step PCR of 35 cycles. The 240-bp PCR product was visualized on agarose gel electrophoresis with ethidium bromide staining. After Southern blotting it was hybridized to a ³²P-labelled oligo probe. All samples were tested 3-6 times and known positive and negative serum samples were analyzed as controls in parallel tests. In order to avoid cross-contaminations, negative controls for each test sample were subjected to RNA extraction, reverse transcription and amplification in parallel test samples. The sensitivity of our test was evaluated by testing several times serial tenfold dilutions of a referenced chimpanzee serum containing 10-6 chimpanzee infectious dose (CID₅₀)/ml equivalent to 3 × 10⁷ genomes/ml (g Eq/ml) [8, 9] (table 1). After hybridization with a ³²P-oligo probe a positive signal can be detected up to the 10-5 dilution of the positive control samples (10 CID₅₀) corresponding to about 10²-10³ g Eq/ml. Therefore the overall sensitivity of our test ranges between 10² and 10³ g Eq/ml.

A score system was used to evaluate semiquantitatively the viremia levels: score 2; samples positive by both agarose gel electrophoresis and blot hybridization (serum HCV-RNA levels higher than 10⁴ g Eq/ml); score 1, samples positive by blot hybridization but negative by agarose gel electrophoresis (HCV-RNA levels ranging between 10³ and 10⁴ g Eq/ml), and score 0, samples negative by both gel electrophoresis and blot hybridization (HCV-RNA less than 10² g Eq/ml).

The high level of specificity and sensitivity of this assay was proved in a recent standardization control trial performed by the European Pathobiologists Group (laboratory 29; Zaaijer et al. [10]). Even though it has recently been claimed that the serum levels of HCV genome can decrease during hemodialysis possibly due to some influence of the dialysis membrane itself [11], we found that viremia higher than 10⁴ g Eq/ml at the beginning of the session did not change during the dialysis course. HCV-RNA was not found in ultrafiltrates and its 100 times concentrates from each patient either at the beginning, during or at the end of the hemodialysis session. HCV-RNA was also repeatedly undetectable on the 4 hemodialysis membranes tested, independent of their constitutive materials (cellulose or polyacrylonitrile derivatives).

HCV has a diameter of 60 nm [8], which largely overcomes the dialysis membrane cutoffs (cuprophane 1.0-1.3 nm, AN 69 5.5 nm). However, since a passage in the dialysis ultrafiltrate has been reported for hepatitis B virus (40 nm) [12], the question of a possible passage of HCV is still open. Indeed, different in vivo and in vitro cutoffs might be considered, as well as subclinical microscopic membrane breaks or a passage of viral fragments instead of total virions.

One important issue which remains unsolved is the real impact of false-positive results in the literature data. It has been shown by quality control trial that this is a major problem in most laboratories. Therefore, a positive RT-PCR result should be repeatedly controlled, at least 3-6 times, and the strictest working conditions should be adopted to avoid contaminations with the amplification products (cDNA) of positive samples tested and previously processed using the same equipment.

We failed to prove the passage of the HCV into the ultrafiltrate, at least under standard conditions. The virus did not show a particular tropism for the dialysis membrane surface as well, since no modifications in viremia were demonstrated and no viral adhesion was found at the end of the session on the dialysis membranes where several circulating proteins and cells are known to adhere. The lack of a significant viral concentration on the dialysis membrane surface reduces the possibility of a favored increased HCV passage in the ultrafiltrate in the case of subclinical breaks.

Our findings do not exclude the possibility of a viral passage in the dialysate during a clinically evident membrane break, but the frequency of this event is in any case much more limited than in the event of transmembrane virus passage.

Moreover, the sporadic passage of HCV in the dialysate after membrane ruptures with consequent dialysis monitor contamination is, according to our findings, of little importance. Indeed the back passage of viruses from dialysate to blood in another patient is theoretically almost impossible, due to the direction of the ultrafiltrate flow. Based on our data, which failed to detect a transmembrane passage of the virus, even the back filtration cannot in any case account for the transmission of HCV infection to the next hemodialysis patient. Theoretically, we cannot exclude that some HCV genome fragments not detectable by our probe can pass through the membrane, but they are devoid of any infectious potential and moreover they would be rapidly destroyed by circulating RNAase.

In conclusion, these findings suggest that under standard conditions current procedures of hemodialysis warrant adequate safety to prevent transmission of HCV. However, the spread of HCV infection by epidemic bouts suggests that other factors could be involved, such as genotypes of HCV, virus load or HCV mutants with particular cellular tropism. These hypotheses and the failure of dialysis equipment should be ruled out by using sentinel units where monthly sera collections, recording the series of patients treated on each machine, are performed in prospective studies.

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Nephron 1996;72:102-103