

Determinant Roles of Environmental Contamination and Noncompliance with Standard Precautions in the Risk of Hepatitis C Virus Transmission in a Hemodialysis Unit

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Background. Nosocomial transmission is the second most frequent cause of hepatitis C virus (HCV) infection. A prospective observational study was conducted to assess the roles of environmental contamination and non-compliance with standard precautions in HCV cross-transmission in a hemodialysis unit.

Methods. Patients undergoing chronic hemodialysis in a French university hospital unit were systematically screened, revealing 2 sporadic cases of HCV transmission. An investigation was launched to determine whether the patients were infected in the hemodialysis unit and the possible roles of environmental contamination and noncompliance with standard precautions. We examined possible relationships among new cases of HCV infection, environmental contamination by blood and HCV RNA, and compliance with guidelines on hand hygiene and glove use.

Results. Two patients experienced seroconversion to HCV during the study period. Phylogenetic analyses showed that 1 of these patients was infected with the same strain as that affecting a chronically infected patient also treated in the unit. Of 740 environmental surface samples, 82 (11%) contained hemoglobin; 6 (7%) of those contained HCV RNA. The rate of compliance with hand hygiene was 37% (95% confidence interval, 35%–39%), and gloves were immediately removed after patient care in 33% (95% confidence interval, 29%–37%) of cases. A low ratio of nurses to patients and poor hand hygiene were independent predictors of the presence of hemoglobin on environmental surfaces.

Conclusion. Blood-contaminated surfaces may be a source of HCV cross-transmission in a hemodialysis unit. Strict compliance with hand hygiene and glove use and strict organization of care procedures are needed to reduce the risk of HCV cross-transmission among patients undergoing hemodialysis.

Hepatitis C virus (HCV) infection is a major health problem. Worldwide, >170 million individuals carry the virus, and the infection becomes chronic in ~80% of adult cases. Approximately 20% of patients with

chronic HCV infection develop cirrhosis, and the incidence of hepatocellular carcinoma is 4%–5% per year in cirrhotic patients [1].

HCV is principally, if not exclusively, transmitted by blood. Historically, the 2 main routes of transmission have been blood transfusion and injection drug use. Since the implementation, in the United States and Europe, of blood-donor screening with highly sensitive EIAs for anti-HCV antibodies and minipool testing for HCV RNA, the incidence of transfusion-transmitted hepatitis C has decreased to ~1 case per 2 million transfused blood units [2, 3]. In France, 3000–4000 new cases of HCV infection still occur every year [4]. Approximately two-thirds of these cases are related to injection drug use, but nosocomial transmission is the

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second most common source of HCV infection. Most cases of HCV transmission in the hospital setting are attributable to patient-to-patient transmission through invasive procedures, such as insertion of an intravascular catheter, colonoscopy, sharing of dialysis equipment, surgery, and sharing of multidose vials [5–11].

The prevalence of HCV infection is high among patients who undergo hemodialysis, because of both contaminated transfusions before the early 1990s and nosocomial transmission. Several outbreaks and sporadic cases of nosocomial HCV or hepatitis B virus transmission in dialysis units have been linked to poor disinfection of dialysis equipment and to poor compliance with standard infection-control measures [9, 12–18]. However, the exact route and mechanism of transmission were unknown in most cases. Here, we examined the intricate roles of noncompliance with standard precautions, environmental contamination, and low nurse-to-patient ratio in cross-transmission of HCV within a dialysis unit.

PATIENTS AND METHODS

Setting and patients. Henri Mondor University Hospital has a 9-bed hemodialysis unit that mainly treats patients with chronic renal failure. A case of HCV seroconversion was detected by systematic screening during the study period. The study period was defined as the interval between the probable date of infection and the discovery of the index case—that is, January–September 2004. Patients' medical files were exhaustively reviewed to eliminate a potential external source of HCV transmission. None of the health care personnel was known to be infected with HCV. No systematic screening of personnel was undertaken. No isolation policy was implemented in the unit. Multidose vials were not in use in the unit.

All patients who undergo regular hemodialysis are screened for anti-HCV antibodies every 3 months, in an effort to detect seroconversion. On 27 July 2004, a case of HCV seroconversion was detected through this screening. To determine whether this case was sporadic or part of an outbreak, all 52 patients with chronic renal failure who were undergoing regular hemodialysis in the unit were tested for anti-HCV antibodies and HCV RNA, as were all patients treated for acute renal failure in the unit during the at-risk period. Six (12%) of the 52 patients (patients 3–8) were known to be chronically infected with HCV, with HCV RNA levels ranging from 4.4 to $>6.9 \log_{10}$ IU/mL at the time of the study. All but 1 of these patients were known to have been infected for several years (e.g., patient 3 has been infected since 2001). A second patient undergoing hemodialysis was found to be HCV RNA positive through culture of a blood sample obtained in July 2004 (tests for anti-HCV antibodies were negative), and an investigation was then launched.

Virological studies and phylogenetic analyses. Anti-HCV antibodies were detected with a third-generation EIA (Vitros

ECi; Ortho-Clinical Diagnostics). We tested for HCV RNA in all patients' blood and in hemoglobin-positive surface swab eluates through use of a sensitive RT-PCR assay (Amplicor HCV, version 2.0; Roche Molecular Systems), with a detection limit of 50 IU/mL.

To estimate the genetic relatedness of HCV strains, 2 HCV genomic regions were PCR amplified and sequenced, including a 328–base pair portion of the nonstructural 5B (NS5B) coding region (nucleotide positions 8271–8597) and the 81–base pair region coding for hypervariable region 1 (HVR1) of the E2 envelope glycoprotein.[19]. HCV genotyping was based on phylogenetic analysis of NS5B sequences, which included prototype sequences of various subtypes of HCV genotypes 1–6. The genetic relatedness of HCV strains was studied by phylogenetic analysis of both the NS5B and HVR1 regions. Sequences were aligned with ClustalW software [20]. Phylogenetic relationships were deduced with the DNADIST-NEIGHBOR module of the Phylogeny Interference Package, version 3.5 [21]. For neighbor-joining analysis, a distance matrix was calculated using a Kimura 2-parameter distance matrix with a transition/transversion ratio of 4.0. Trees were drawn with TREEVIEW or NJ-Plot programs [22]. Their robustness was assessed by bootstrap analysis of 1000 replicates with the SEQBOOT module of the Phylogeny Interference Package program.

The index patient (patient 1) experienced HCV seroconversion in July 2004. The second case of HCV seroconversion during the study period (patient 2) was identified by systematic screening for HCV RNA. To determine whether chronically infected patients were the source of the new cases, the sequences of 2 HCV genomic regions, including a portion of the NS5B coding sequence and the sequence coding for HVR1, were compared among the 8 infected patients, relative to reference sequences. Phylogenetic analyses of the NS5B region (figure 1) and the HVR1 (figure 2) showed that newly infected patient 2 was infected with the same HCV genotype 1 strain as was chronically infected patient 3. In contrast, patient 1 was infected with an HCV genotype 3a strain that was unrelated to the strains infecting the other 6 chronically infected patients (all infected with genotype 1). Despite the proximity of the HCV strains from patients 4–8 in the NS5B phylogenetic tree (figure 1), HVR1 analysis showed that those patients were infected with unrelated strains (figure 2).

Thus, 2 patients were infected during the at-risk period, 1 of whom (patient 2) was infected with the same strain as was a chronically infected patient (patient 3). The other newly infected patient (patient 1) was infected with a genotype 3a strain, which could have been acquired either from a patient occasionally treated in the dialysis unit or from an external source.

Risk factors of HCV transmission. Potential risk factors of HCV transmission were hypothesized—namely, contamination of dialysis equipment (through machine sharing and inadequate

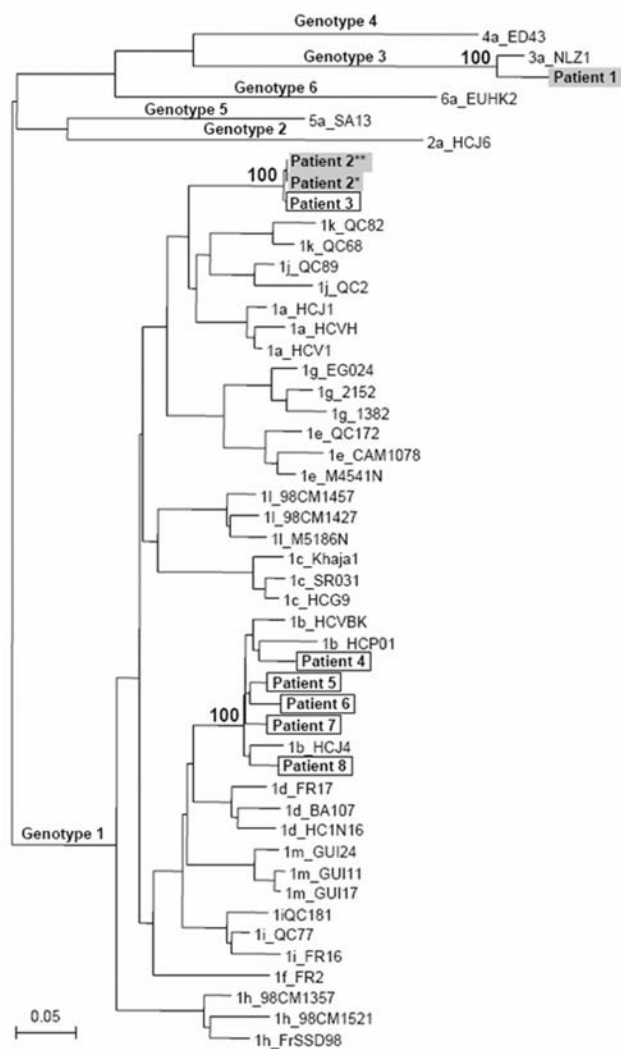


Figure 1. Phylogenetic tree plotted with nonstructural 5B sequences (nucleotide positions 8271–8597) from the 2 patients recently infected in our hemodialysis unit (patients 1 and 2; shaded boxes), the 6 patients known to be chronically infected with hepatitis C virus (HCV) and regularly treated in our hemodialysis unit (patients 3–8; unshaded boxes), and reference HCV strains of different genotypes (the type and subtype are indicated just before the strain identification letters and/or numbers). Two samples were available and were included in the analysis for patient 2, *July 2004; **September 2004. Nucleotide sequence of the nonstructural 5B gene of HCV-ED43 was used as an outgroup root.

environmental disinfection), noncompliance with standard precautions, and variation of the nurse-to-patient ratio in the hemodialysis unit.

The use and maintenance of dialysis equipment was reviewed by the local infection control team according to the written local procedures that are based on published data and recommendations. Dialyzers were not being reused, and dialysis machines (AK100; Gambro) were disinfected after each session, according to a written protocol combining chemical (peroxy-

acetic acid [Dialox]) and sodium hypochlorite) and heat disinfection.

Surfaces at risk of contamination with infected blood were defined as the most frequently manipulated surfaces—including dialysis machines, shared waste carts, patients' removable tables, and work benches. At-risk surfaces were swabbed during dialysis sessions (30 swabs per day on 25 consecutive days) on a surface area of ~100 cm², by using a cotton swab moistened with sterile distilled water that was then eluted in 1 mL of sterile distilled water. Hemoglobin was detected with reagent strips (Hemastix; Bayer HealthCare) with a detection limit of 150 µg Hb/L—that is, the equivalent of 5 erythrocytes per microliter. All hemoglobin-positive samples were tested for HCV RNA [23, 24].

Compliance with standard precautions (hand hygiene and glove use) was studied in the dialysis unit each day for three 30-min periods—during the morning, afternoon, and night shifts—for 7 weeks (2 weeks during September 2004 immediately after the first case alert and 5 weeks during June–July 2005). All staff categories were studied, in an open, unobtrusive manner, by 5 specially trained members of the infection control team, with use of a standardized questionnaire [25]. Hand hygiene opportunities tailored to the care activities in the hemodialysis unit were listed in the questionnaire (i.e., before and

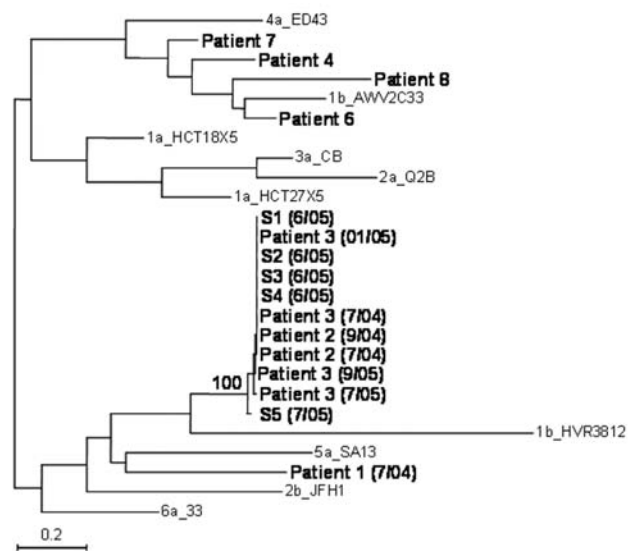


Figure 2. Phylogenetic tree plotted with hypervariable region (HVR) 1 sequences from the 6 patients known to be chronically infected with hepatitis C virus (HCV) and regularly treated in our hemodialysis unit (patients 3–8), including the patient who transmitted HCV to patient 2 (i.e., patient 3), the 2 patients infected in our hemodialysis unit (patients 1 and 2), the 5 environment surfaces that tested positive for HCV RNA and that could be PCR amplified in that region (S1–S5), and reference HCV strains of different genotypes (the type and subtype are indicated just before the strain identification letters and/or numbers). Dates of sampling are shown in parentheses.

after central venous catheter or fistula handling; preparation of material, connection, disconnection, dressing, and manipulation of lines and before and after direct contact with a patient; handling of other invasive devices, if present; measurement of temperature; measurement of arterial pressure; etc.). The handling of catheter and fistula were considered to be activities with high risk of HCV transmission. Overall, 2382 opportunities were observed during 197 shifts, with a total of 98 h of observation.

Glove use was observed during the same periods as was hand hygiene. For each care activity, the following variables were collected on the same standardized questionnaire as that used for hand hygiene: type of contact, wearing gloves during contact, and glove removal immediately after contact. Wearing gloves is recommended in the unit when exposure to body fluids is anticipated.

With consideration that the nurse-to-patient ratio (including nurses and nurse assistants) may influence the risk of HCV transmission, the ratio was recorded during each observation period, and the average nurse-to-patient ratio per shift (morning, afternoon, and night) was determined by calculating the median ratio for all the relevant observation periods. Hand hygiene compliance was also calculated for each of the 3 shifts.

Statistical analysis. Percentages and 95% CIs were calculated. The χ^2 test or Fisher's exact test was used, as appropriate, to compare proportions. The Mann-Whitney nonparametric test was used to compare continuous variables. Each potential risk factor for environmental hemoglobin contamination (i.e., nurse-to-patient ratio and hand hygiene compliance) was tested in a univariate model, and results were then entered in a logistic regression model. Variables were not dichotomized. To take into account the interdependence of observations made during the same shift, we used robust estimates of variance (generalized estimating equations) in which each shift observation was included as a cluster. Goodness of fit was assessed using the Hosmer-Lemeshow χ^2 test, and discrimination was determined from the area under the receiver operating characteristics curve. Accuracy was considered to be good when the area under the receiver operating characteristics curve had a range of 0.70–0.80 and was considered to be excellent when it was >0.80. The adjusted OR and 95% CI were calculated for each factor that was statistically significant in the logistic regression model. *P* values <.05 were considered to be statistically significant. All tests were 2 tailed. Statistical tests were performed using Intercooled Stata software, version 8.2 (Stata).

RESULTS

Virological study of environmental surfaces. A total of 740 surface samples were collected in the dialysis unit during June–August 2005, comprising 663 (90%) from dialysis machines

and 77 (10%) from other surfaces (table 1). Hemoglobin was found in 82 samples (11%), including 71 (10%) from surfaces where blood was not evident. Among the 25 hemoglobin-positive samples collected from dialysis machines, 5 had been obtained after external disinfection of the machine. Six (7%) of the 82 hemoglobin-positive samples contained detectable levels of HCV RNA, comprising 4 samples taken from a dialysis machine and 2 from a shared waste cart (table 1). The HVR1-coding region could be PCR-amplified and sequenced in 5 of these 6 samples, designated S1–S5. These sequences were compared with HVR1 sequences recovered from patients 1–8 during the at-risk period (except for patient 5, in whom HVR1 could not be amplified) and also from patient 3 at the time of surface sampling (figure 2). As shown in figure 2, phylogenetic analysis revealed that all sequences found in environmental samples were closely related to those isolated from patient 2 when he was infected in 2004 and to those from patient 3, from whom samples were obtained both in 2004 and in 2005. Note also in figure 2 the very slow genetic evolution of the HVR1 in patient 3 (only 4 nucleotide substitutions accumulated in 14 months; data not shown), probably because of hemodialysis-associated immune suppression. Interestingly, the same HCV strain was isolated from 2 environmental samples taken at a 6-h interval from the same machine that had been used to treat 2 different patients.

Assessment of practices. Compliance with local precautions for machine use and internal disinfection was adequate. Multidose vials were never shared between patients. The finding that patients 2 and 3, who were infected with closely related HCV strains (figures 1 and 2), had always undergone dialysis during the same sessions but had never shared the same machine strongly suggested that patient 2 had been infected by patient 3 via the hands of a health care worker.

Compliance with standard precautions during the investigation is shown in figure 3. Overall, 2382 opportunities for hand hygiene were observed (2358 [99%] for nurses; 24 [1%]

Table 1. Environmental samples containing hemoglobin and/or hepatitis C virus (HCV) RNA.

Sample site	No. of samples	Positive samples, no. (%)	
		Hemoglobin	HCV RNA
Dialysis machine	663	36 (5)	4 (11)
Shared waste cart	27	24 (89)	2 (8)
Patients' removable table	9	6 (67)	0 (0)
Miscellaneous ^a	41	16 (39)	0 (0)
Total	740	82 (11)	6 (7)

NOTE. HCV RNA–positive findings are percentages of the number of hemoglobin-positive samples.

^a Including nursing preparation area, wheelchairs, and patient file cart.

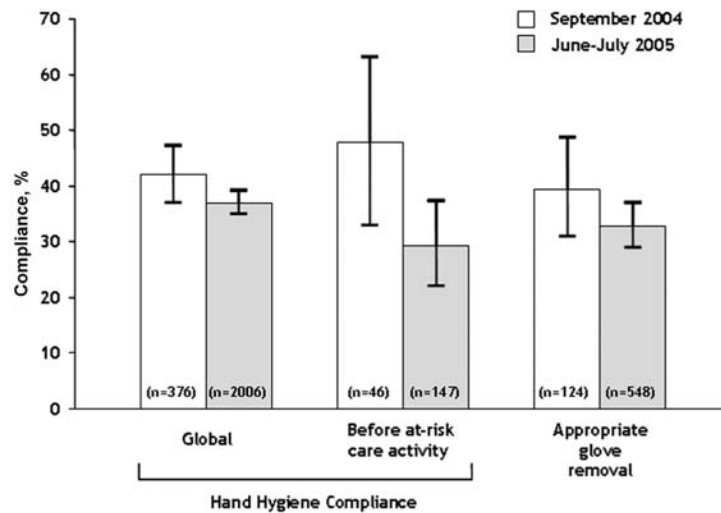


Figure 3. Compliance with guidelines for health care worker hand hygiene and appropriate glove use during dialysis. At-risk care activities consisted of handling dialysis catheters or fistulas. Whiskers, 95% CIs.

for nurse assistants). Immediately after the infection alert (September 2004), compliance with hand hygiene immediately before handling a dialysis catheter or fistula was significantly higher ($P < .001$) than it was several months later (figure 3). Globally, gloves were worn in 857 (36%) of observed contacts with a patient or the environment. When worn, gloves were removed immediately after a contact in only 672 (34.1%) of cases (95% CI, 30.5%–37.8%). There was no statistically significant difference between the findings of the 2 periods of observation. As shown in table 2, a low nurse-to-patient ratio and a poor rate of hand hygiene compliance were independently associated with the detection of hemoglobin on environmental surfaces.

DISCUSSION

Several reports of nosocomial HCV transmission in the hemodialysis setting have been published, but the investigations were incomplete and the routes of transmission remained unclear [13, 17, 18, 26]. Allander et al. [26] reported nosocomial HCV transmission in a series of patients who underwent dialysis at the same time but who did not share dialysis equipment. Those authors postulated, but did not show, that the environment was contaminated. Compliance with standard precautions was not studied.

To our knowledge, ours is the first study to demonstrate that a low nurse-to-patient ratio and poor compliance with guidelines for hand hygiene and glove use are independent predictors of environmental contamination by blood and HCV. By combining genetic and phylogenetic analyses of HCV recovered from patients' blood and the environment with measurements of compliance with standard precautions, we showed that: (1)

2 sporadic cases of HCV transmission occurred in the dialysis unit during the study period, 1 of which was unequivocally due to patient-to-patient transmission within the unit; (2) the dialysis environment was frequently contaminated by blood, including HCV-infected patients' blood, as shown by the detection of hemoglobin, sometimes associated with detectable levels of HCV RNA in a substantial proportion of swabs; and (3) compliance with guidelines for hand hygiene and glove use during patient care was poor, raising the possibility of HCV transmission via the hands of health care workers. Interestingly, all HCV-infected blood found in environmental samples belonged to the patient who indirectly infected another patient undergoing dialysis.

In our study, hemoglobin was found in 11% of environmental samples, and 7% of those positive samples contained detectable HCV RNA. Hepatitis B virus transmission has been linked to the presence of the virus on environmental surfaces—in the absence of visible blood [27]. Hepatitis B virus has been reported to remain viable on environmental surfaces for at least 7 days at room temperature [28, 29]. HCV RNA has been shown to be resistant for at least 48 h on inert surfaces at room temperature [24, 30, 31]. A robust cell culture system for HCV was recently developed, but it cannot be infected with viruses other than those produced after cell culture transfection of a specific HCV clone [32–34]. Cell culture systems that can be directly infected by HCV-infected patients' blood will be needed to determine how long HCV remains infective in the environment. Even in the absence of such data, our results strongly suggest that infectious HCV is present in the dialysis environment and that HCV can be transmitted by the hands of health care workers. We did not, however, sample health care workers'

Table 2. Factors independently associated with environmental blood contamination during nursing shifts.

Variable	Univariate analysis of environmental hemoglobin, by daily shifts		Multivariate analysis	
	Hemoglobin found (n = 28)	Hemoglobin not found (n = 14)	OR (95% CI)	P
Nurse-to-patient ratio, mean ± SD	0.55 ± 0.23	0.78 ± 0.50	0.03 (0.002–0.39)	.008
Hand hygiene compliance, mean % ± SD	39 ± 15	44 ± 17	0.93 (0.88–0.99)	.036

NOTE. Performance of the model, Hosmer-Lemeshow goodness-of-fit; $P = .386$; area under receiver operating characteristics curve, 0.768.

gloved or ungloved hands during care activities, because this would have hindered the assessment of compliance with standard precautions by increasing the Hawthorne effect.

The rate of compliance with standard precautions in our study was similar to that reported elsewhere about a similar setting [35, 36]. A recent survey of hand hygiene practices in 9 Spanish hemodialysis units showed poor compliance, both before and after contact with patients (14% and 36%, respectively) [36].

Permanent glove use can impair compliance with hand hygiene [37] and may thus lead to cross-transmission of infectious agents. This is the first time that glove use and removal have been studied in relation to the risk of environmental contamination. Gloves are worn mainly for health care worker self-protection, rather than to prevent patient cross-infection. The recommendation that gloves always be worn in the hemodialysis setting, whatever the type of contact (environment or patient) [38], therefore, may be confusing and may expose patients to HCV transmission if not followed properly, with systematic glove removal and hand hygiene between care procedures.

We found that a nurse-to-patient ratio <0.60 was independently associated with hemoglobin contamination of environmental surfaces. Understaffing is a recognized major risk factor for nosocomial infection [39–41]. Recently, a Brazilian study of 22 dialysis centers showed that the number of patients per health care worker was independently related to the risk of hepatitis B virus infection [16]. Petrosillo et al. [42] showed, in a prospective multicenter study in Italian hemodialysis units, that a low staff-to-patient ratio is an independent predictor of the risk of HCV nosocomial transmission. Therefore, to limit the spread of blood in the dialysis environment, we recommend that at-risk care procedures, such as connection and disconnection of equipment to the patient, be performed by a pair of nurses: one working with the patient and the other working with the machine.

In conclusion, blood-contaminated surfaces may represent a source of HCV transmission, via health care workers' hands or gloves. Environmental contamination is mainly a consequence of poor adherence to standard precautions in the hemodialysis setting. Strict adherence to guidelines for hand hy-

giene and glove use and strict organization of care procedures, with an adequate nurse-to-patient ratio, should help to reduce the risk of environmental contamination and, thus, HCV transmission in patients undergoing dialysis.

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Potential conflicts of interest. All authors: no conflicts.

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