

## TRANSMISSION OF HEPATITIS C VIRUS BY A CARDIAC SURGEON

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**Abstract Background.** In the course of a study conducted from 1992 through 1994 of the efficacy of screening blood donors for antibodies to hepatitis C virus (HCV), we found that two patients had acquired hepatitis C after cardiac surgery, with the transmission apparently unrelated to blood transfusions. Because their surgeon had chronic hepatitis C, we sought to determine whether he was transmitting the virus to his patients.

**Methods.** Of 222 of the surgeon's patients who participated in studies of post-transfusion hepatitis between 1988 and 1994, 6 contracted postoperative hepatitis C, despite the use of only seronegative blood for transfusions. All six patients had undergone valve-replacement surgery. Analyses were performed to compare nucleotide sequences encompassing the hypervariable region at the junction between the coding regions for envelope glycoproteins E1 and E2 in the surgeon, the patients, and 10 controls infected with the same HCV genotype.

**Results.** The surgeon and five of the six patients with

hepatitis C unrelated to transfusion were infected with HCV genotype 3; the sixth patient had genotype 1 and was considered to have been infected from another source. Thirteen other patients of the surgeon had transfusion-associated hepatitis C and were also infected with genotype 1. The average net genetic distance between the sequences from the five patients with HCV genotype 3 and those from the surgeon was 2.1 percent (range, 1.1 to 2.5 percent;  $P < 0.001$ ), as compared with an average distance of 7.6 percent (range, 6.1 to 8.3 percent) between the sequences from the patients and those from the controls. The results of a phylogenetic-tree analysis indicated a common epidemiologic origin of the viruses from the surgeon and the five patients.

**Conclusions.** Our findings provide evidence that a cardiac surgeon with chronic hepatitis C may have transmitted HCV to five of his patients during open-heart surgery. (N Engl J Med 1996;334:555-60.)

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SCREENING of blood donors for antibodies to hepatitis C virus (HCV) has reduced the incidence of transfusion-associated hepatitis C. Use of the first enzyme immunoassays for screening was associated with an 80 percent reduction in the risk of infection among transfusion recipients in several retrospective<sup>1,2</sup> and prospective<sup>3,4</sup> studies. A further reduction in the risk is anticipated with newer enzyme immunoassays for antibodies to HCV.<sup>1,4,5</sup> Some studies<sup>6-8</sup> have found no HCV infections among recipients of blood screened with these second-generation enzyme immunoassays.

During a prospective investigation of the efficacy of second-generation immunoassays for HCV antibodies in preventing post-transfusion hepatitis C, we identified two patients who had contracted acute hepatitis C six to eight weeks after open-heart surgery. Repeated serologic and virologic testing of the donors of blood given to these patients failed to identify any with HCV infection. Further epidemiologic investigation linked both cases to a cardiac surgeon known to have chronic hepatitis C. We report epidemiologic and molecular evidence that this surgeon may have transmitted HCV to at least five patients between 1988 and 1993.

### METHODS

#### Identification of Former Patients of the Surgeon

As part of the Barcelona Post-Transfusion Hepatitis Study,<sup>4,9</sup> we conducted two consecutive, prospective studies between 1988 and

1990 involving 525 patients undergoing cardiac surgery. For 7 of 30 patients with postoperative hepatitis C, retrospective immunoassays for HCV antibodies in all sets of blood units used for transfusions failed to identify any seropositive units. All but one of the seven patients underwent surgery at the same institution. During another study of HCV screening for patients undergoing heart surgery, conducted between 1992 and 1994, two additional patients with hepatitis C unrelated to transfusions were identified; their surgeon had chronic hepatitis C. All former patients of this surgeon who participated in the two studies were identified. The surgeon's operative logs for the years 1988 through 1994 were reviewed and cross-matched with the lists of participants in the three studies who had completed six months of follow-up. The study protocol was reviewed and approved by the bioethics committee of Hospital General Universitari Vall d'Hebron.

#### Detection, Titration, Genotyping, and Sequencing of HCV RNA

Nucleic acids were extracted from plasma or serum and tested for HCV RNA by the polymerase chain reaction (PCR) with the use of a commercial detection kit (Amplicor HCV, Roche Molecular Diagnostic Systems, Branchburg, N.J.).<sup>10</sup> Quantitation of HCV RNA was performed with a commercial branched-chain-DNA signal-amplification assay (Quantiplex HCV, Chiron, Emeryville, Calif.).<sup>11</sup>

We determined the HCV genotype in samples from the surgeon, all his former patients who had contracted hepatitis C and their corresponding HCV-infected blood donors, and a group of 150 consecutive blood donors in Barcelona who were positive for HCV antibodies. Genotyping was performed with a commercial probe-hybridization assay (InnoLipa HCV, Innogenetics, Ghent, Belgium), which has been shown to identify accurately HCV genotypes 1 through 6,<sup>12</sup> according to the classification proposed by Simmonds et al.<sup>13</sup>

A sequence analysis was performed with 188-nucleotide fragments encompassing the first hypervariable region (HVR-1) at the junction between the coding regions for envelope glycoproteins E1 and E2 (Fig. 1) in viral isolates from the surgeon, the five patients infected with HCV genotype 3, and the five blood donors infected with HCV genotype 3 from whom sufficient samples were available. The fragments were amplified by PCR with the use of primers developed on the basis of published sequences.<sup>14</sup> The PCR products were cloned and sequenced as previously described.<sup>15</sup> Two to five clones were sequenced from each sample. The E1-E2 fragment was chosen for sequence analysis because the HVR-1 domain exhibits a sufficiently high degree of variability to distinguish between HCV isolates of the

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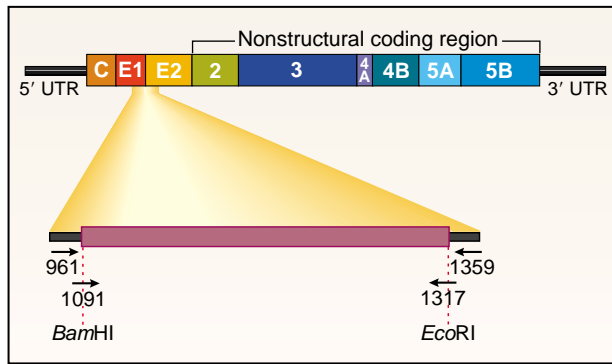


Figure 1. Organization of the HCV Genome Showing the Putative Structural-Protein Coding Regions (Core [C] and Envelope [E1 and E2] Glycoproteins) and Nonstructural-Protein Coding Regions (2, 3, 4A, 4B, 5A, and 5B).

Amplified products at the E1–E2 junction are depicted with their respective ends indicated by the corresponding nucleotide positions according to the HCV type 1 prototype. The purple bar represents the restriction fragment generated for subsequent cloning. The arrows indicate the outer primers (5'CGAGAA-TTCAAGGACATCCAGTAGAGTTGAACT3' and 5'TCCCGGAT-CCGGATATGATGATGAATTGGT3'; nucleotide 961 to 1359) and inner primers (5'AGCGAATTC AAGCTATGAACCCGGTGT-TTAT3' and 5'TTTGGATCCTGCAAGGCAACTGGGCCAAGG-T3'; nucleotide 1091 to 1317) used for complementary DNA synthesis and nested-PCR amplification, synthesized according to conserved representative sequences of the HCV-3a genotype.<sup>14</sup> UTR denotes untranslated region.

same subtype<sup>16,17</sup> and has been used in previous studies of infections known to be epidemiologically linked.<sup>18–20</sup> The sequences have been submitted to the European Molecular Biology (EMBL) Bank (Heidelberg, Germany; accession numbers Z68708 through Z68745).

Sequences from the surgeon, patients, and local blood donors, as well as consensus sequences corresponding to the E1–E2 region from five genotype 3a isolates available from the EMB data bank (CENS1.3a, NZL15.3a, US114.3a, TH855.3a, and HEW235.3a), were included in the sequence analysis.

### Comparison of HCV Sequences

After the E1–E2 sequences had been aligned, nucleotide distances were estimated by generating a distance matrix based on all pairwise comparisons of sequences.<sup>21,22</sup> The matrix was calculated with the use of Kimura's two-parameter model, which assumes a different probability for transitions (purine-to-purine or pyrimidine-to-pyrimidine changes) and transversions (purine-to-pyrimidine or pyrimidine-to-purine changes).<sup>22</sup> On the basis of the comparison of aligned sequences, we set the transition:transversion ratio at 1.3, because there were 1.3 times as many transitions as transversions. We defined the genetic distance between the viral isolates from one person and those from another person or group as the net nucleotide distances within and between groups of sequences, according to the procedure of Nei and Jin.<sup>23</sup> To examine the full range of relations among the viral isolates from the surgeon, his patients, and the controls, we performed phylogenetic-tree analyses of the E1–E2 nucleotide sequences.<sup>22</sup> The trees were constructed by clustering the matrix of pairwise distances with the use of the neighbor-joining algorithm<sup>24</sup> and by using nucleotide sequences directly to generate a maximum-likelihood tree based on a random model of molecular evolution.<sup>21,22</sup>

### Medical and Surgical Practices of the Surgeon

The medical records of the surgeon were reviewed by one of us. The surgeon was interviewed on several occasions by three of us and by the chief of cardiac surgery to review his operating-room procedures and compliance with infection-control practices. In addition, the surgeon completed a questionnaire covering the frequency and

nature of percutaneous injuries, history of cutaneous diseases, frequency of glove changing, and differences in the risk of injuries according to whether he was acting as primary surgeon or assisting other surgeons. Interviews were conducted with other members of the cardiac-surgery team, the operating-room supervisor, and the anesthesiologists, perfusionists, and nurses who worked regularly with the surgeon.

### Statistical Analysis

Continuous variables are presented as means  $\pm$  SE. The chi-square test or Fisher's exact test was used to compare categorical variables. Wilcoxon's rank-sum test was used to compare genetic distances between viral isolates. The maximum-likelihood tree was generated by a DNA-sequence maximum-likelihood analysis performed with a computer program (Phylogeny Inference Package, version 3.5c, provided by J. Felsenstein, Department of Genetics, University of Washington, Seattle).<sup>21</sup> The probability associated with a given distance between branches or nodes of the tree was estimated with a likelihood-ratio test, by comparing the likelihood of the tree with and without the corresponding branch. All P values are two-sided, and a value less than 0.05 was considered to indicate statistical significance. The stability of the observed topology of a tree was assessed by the bootstrap method, in which new data sets were obtained by drawing random samples of observations from the original data.<sup>22</sup> We used 500 of these bootstrap samples. If more than 70 percent of the trees constructed from resampled data sets were essentially similar to the tree constructed from the original data set, the topology was considered stable.

## RESULTS

### Identification of Former Patients of the Surgeon

Between August 1988 and June 1994, the surgeon had performed or assisted with surgery in 643 patients, of whom 222 (35 percent) had participated in one of the three prospective follow-up studies in the Barcelona Post-Transfusion Hepatitis Study. Eighty percent of the procedures for which the physician was the primary surgeon were valve replacements. Of the 222 patients, 19 (8.6 percent) had contracted acute hepatitis C 6 to 12 weeks after the surgery. Thirteen of these 19 patients had received blood from at least one donor known to be positive for HCV antibodies.<sup>4,9</sup> Serum samples from all sets of blood units used for transfusions in the remaining six patients were negative for HCV antibodies by second- and third-generation immunoassays on at least three occasions (at the time of the donation and 6 to 12 months later).<sup>4</sup>

The characteristics of the six patients with hepatitis C unrelated to transfusions are shown in Table 1. Five patients were treated with interferon alfa-2b (Intron A, Schering, Kenilworth, N.J.). Four of the five patients remained in biochemical and virologic remission for 18 to 75 months,<sup>25</sup> and one had a relapse.

### HCV Genotyping and Comparison of Sequences

The HCV isolates from the 13 patients with transfusion-associated hepatitis C and from their corresponding HCV-infected donors were all genotype 1. Of the 150 HCV-infected blood donors, 126 had genotype 1, 7 had genotype 2, 10 had genotype 3, 6 had genotype 4, and 1 had genotype 5. In contrast, the surgeon and five of the six patients with hepatitis C unrelated to transfusions (Patients 1, 2, 4, 5, and 6) were infected with HCV genotype 3. Patient 3, who had genotype 1,

Table 1. Characteristics of the Six Patients with Acute Hepatitis C Unrelated to Transfusions.

PATIENT NO.	AGE (YR)	SEX	TYPE OF SURGERY	DATE OF SURGERY (MO/YR)	DATE OF SAMPLE USED FOR SEQUENCING (MO/YR)	INCUBATION PERIOD (WK)	INTERVAL TO SERO-CONVERSION (WK)	NATURE OF INFECTION	INTERFERON ALFA-2b TREATMENT		HCV GENOTYPE
									DOSE	OUTCOME	
1	73	M	Valve replacement	11/88	1/89	8	14	Chronic	3 million units twice a week for 6 months	Response followed by relapse	3
2	23	M	Valve replacement	2/89	4/89	6	18	Chronic	3 million units twice a week for 12 months	Sustained response	3
3	57	F	Valve replacement	3/89	6/89	12	16	Chronic	Untreated	—	1*
4	59	F	Valve replacement	5/89	7/89	8	8	Acute	3 million units twice a week for 12 weeks	Sustained response	3
5	66	F	Valve replacement	11/92	12/93†	6	12	Chronic	3 million units twice a week for 12 months	Sustained response	3
6	38	M	Valve replacement	7/93‡	9/93	6	8	Chronic	3 million units twice a week for 12 months	Sustained response	3

\*The patient was considered to have infection from another, undetermined source and was excluded from further analysis.

†By mistake, the sample used for sequencing was obtained one year after infection, just before interferon treatment; it was not an acute-phase sample.

‡The day after the initial procedure, the patient underwent emergency surgery to reposition a blocked aortic-valve prosthesis.

was considered to have been infected from another source and was therefore excluded from the analysis.

Net nucleotide distances between the viruses isolated from the five patients (Patients 1, 2, 4, 5, and 6) and those isolated from the controls ranged from 6.1 to 8.3 percent (average, 7.6 percent [1 percent equals 1.88 nucleotides]), whereas the net distances between the isolates from the patients and those from the surgeon ranged from 1.1 to 2.5 percent, with an average of 2.1 percent ( $P < 0.001$ ) (Table 2). The latter distances are similar to those reported for HCV infections with known epidemiologic links.

#### Phylogenetic-Tree Analysis

The maximum-likelihood and neighbor-joining procedures generated trees with similar topology in which all sequences from the five patients and from the surgeon clustered together in a monophyletic nest (Fig. 2). This finding is consistent with a common epidemiologic origin. In the maximum-likelihood tree, the distance between the nodes or branches separating the sequences from the surgeon and the control sequences was 0.05 (95 percent confidence interval, 0.01 to 0.08;  $P < 0.001$ ). A distance of 0.05 means a genetic distance or divergence of 5 nucleotide substitutions per 100 nucleotide sites. The results with the neighbor-joining procedure are shown in Figure 2. A similar clustering of sequences in isolates from the patients and the surgeon was observed in 448 of 500 bootstrap samples (89.6 percent). In both trees, the sequences from the surgeon were distributed less uniformly than those from the patients, with some of the sequences from the surgeon clustering closer to sequences from the patients than to other sequences from the surgeon.

#### Medical Evaluation of the Surgeon

The surgeon reported having sustained a major percutaneous injury with a scalpel in 1984, while operating on a person who was found to have chronic infection with the hepatitis B virus (HBV). A serum sample obtained

from the surgeon after the accident was negative for hepatitis B surface antigen, with a normal alanine aminotransferase level. He was given hepatitis B immune globulin and the hepatitis B vaccine (Engerix-B, SmithKline Beecham, Philadelphia). Six months later, his serum alanine aminotransferase level was found to be elevated.

In December 1991, the surgeon was evaluated because of persistently elevated alanine aminotransferase levels (360 U per liter; normal range, 7 to 34). The HCV RNA level was 22 million genome equivalents per milliliter. Serologic tests for anti-HCV antibodies were positive, and he was started on interferon alfa-2b therapy (Intron A), at a dosage of 3 million units subcutaneously three times a week for 11 months. He had a partial response, followed by a rapid relapse, with alanine aminotransferase and HCV RNA levels returning to pretreatment values.

Table 2. Mean ( $\pm$ SE) Net Nucleotide Distances within and between Groups of E1–E2 Sequences in Viral Isolates from the Surgeon, 5 Patients, and 20 Controls.\*

PERSON	NO. OF SEQUENCED CLONES	INTRAPERSON NUCLEOTIDE DISTANCE	INTERPERSON NUCLEOTIDE DISTANCE	
			BETWEEN PATIENT AND SURGEON	BETWEEN PATIENT AND CONTROLS
<i>no. of substitutions per 100 nucleotide sites</i>				
Surgeon	5	0.05 $\pm$ 0.01	—	0.07 $\pm$ 0.02
Patient 1	3	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.08 $\pm$ 0.02
Patient 2	2	0.04 $\pm$ 0.02	0.02 $\pm$ 0.01	0.08 $\pm$ 0.02
Patient 4	3	0.00 $\pm$ 0.00	0.02 $\pm$ 0.01	0.08 $\pm$ 0.02
Patient 5	3	0.06 $\pm$ 0.01	0.01 $\pm$ 0.00	0.06 $\pm$ 0.02
Patient 6	2	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.09 $\pm$ 0.02
Controls	20†	0.20 $\pm$ 0.02	—	—

\*The net nucleotide distance can also be expressed as the percentage of distance or divergence (see the Methods section).

†The 20 control clones included 15 from blood donors infected with HCV genotype 3 (2 to 4 clones per donor) and 5 published HCV genotype 3a consensus sequences.<sup>14</sup>

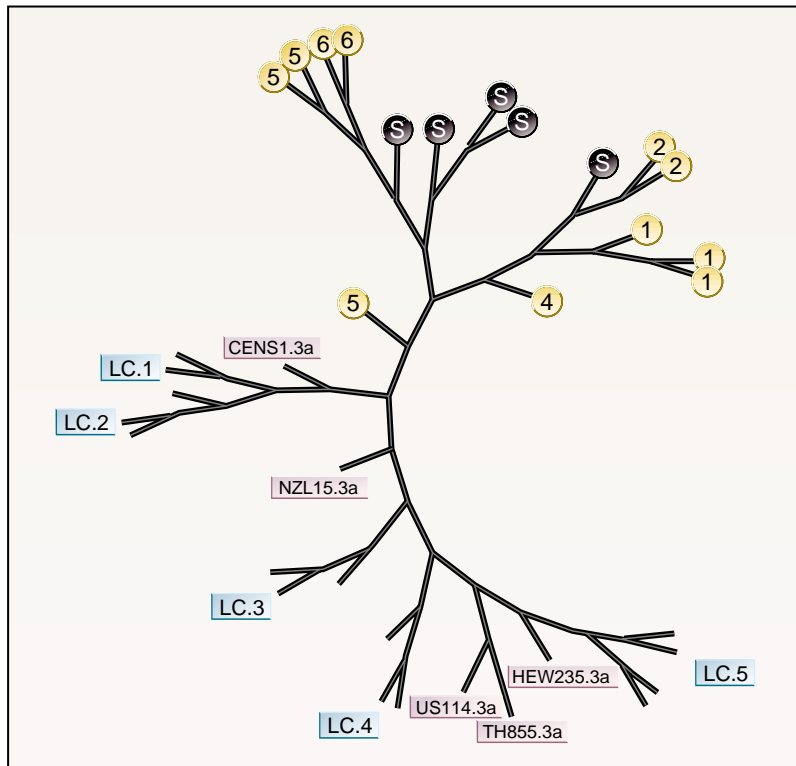


Figure 2. Neighbor-Joining Phylogenetic-Tree Analysis Comparing Coding Sequences in HCV Hypervariable Region 1 in Viral Isolates from the Surgeon (S), Five Patients (1, 2, 4, 5, and 6), Local Controls (LC.1 through LC.5), and Controls from the EMB Data Bank (CENS1.3a, NZL15.3a, US114.3a, TH855.3a, and HEW235.3a).

All sequences from the five patients and the surgeon clustered together in a monophyletic nest. The clustering of sequences from the patients and the surgeon was similar in 448 of 500 bootstrap analyses (89.6 percent). Each branch of the tree corresponds to a different cloned sequence (e.g., there are five branches for the surgeon because five of his cloned sequences were analyzed), except for Patient 4, whose single branch corresponds to three clones with identical nucleotide sequences.

He continued performing surgery until November 1994, when he was informed that preliminary data suggested he might have transmitted HCV to some of his patients. He voluntarily stopped performing invasive procedures and was given interferon alfa-2b (3 million units subcutaneously three times a week), plus ribavirin (ICN Pharmaceuticals, Costa Mesa, Calif.; 1200 mg orally every day), which resulted in normalization of his liver-enzyme levels and clearance of serum HCV RNA as assessed by PCR within two months. After two additional months, during which viremia was not detected, he was allowed to resume the practice of surgery. The surgeon was tested weekly for HCV RNA during the first two months of treatment and every other week for an additional two months. He is still being treated (as of December 1995, he had been treated for 11 months), and his serum is tested once a month. He remains in complete remission.

#### Incidence of Hepatitis C among Patients

Table 3 shows the incidence of hepatitis C among the 222 patients who underwent procedures performed by

the surgeon or in which he assisted and who participated in the prospective follow-up studies. As expected, the 13 transfusion-associated cases were all observed between 1988 and 1989, before the implementation of screening of blood donors for anti-HCV antibodies. The occurrence of these cases was not associated with the surgeon's role during the operation. The cases unrelated to transfusions occurred in 5 of 84 patients undergoing procedures performed by the surgeon but in none of 134 undergoing procedures in which he assisted ( $P=0.008$ ).

#### Percutaneous Injuries

The surgeon reported an overall incidence of about 20 percutaneous injuries per 100 procedures. Most of these injuries occurred in the course of tying the wires during closure of the sternum; in many cases, he did not notice the injury until after the procedure. He regularly performed the sternotomy closure himself when acting as primary surgeon but only occasionally when assisting. He reported an incidence of 2 percutaneous injuries with sharp objects and needles per 100 procedures. After such an injury, he customarily removed the instrument from the field and changed gloves.

The surgeon had no history of dermatitis and did not recall any occasion when he had bled into a patient's wound. Interviews with other members of the operating teams corroborated the surgeon's recollections and confirmed that whenever a percutaneous injury had occurred, gloves had been changed, and the instrument immediately removed from the field. Two fellow surgeons who completed the same questionnaire also acknowledged frequent percutaneous injuries while closing the sternum with wires.

#### DISCUSSION

Our findings provide evidence that a cardiac surgeon with chronic hepatitis C may have transmitted HCV to five of his patients during open-heart surgery. Transmission of HCV from infected patients to health care workers has been documented.<sup>26-29</sup> There have also been reports of the transmission of other blood-borne viruses, such as HBV and the human immunodeficiency virus (HIV), from infected health care workers to patients.<sup>30-33</sup>

Our study could not pinpoint the circumstances and

Table 3. Incidence of Acute Hepatitis C (Transfusion-Associated and Non-Transfusion-Associated Cases) among 222 Patients of the HCV-Infected Surgeon.\*

DATE AND SURGEON'S ROLE DURING PROCEDURE	TOTAL NO. OF PATIENTS	NO. FOLLOWED PROSPECTIVELY (%)	NO. WITH POSTOPERATIVE HEPATITIS C	
			RELATED TO TRANSFUSION	UNRELATED TO TRANSFUSION
1988				
Surgeon	31	12 (39)	2	1
Assistant	32	15 (47)	2	0
1989				
Surgeon	39	29 (74)	3	2
Assistant	81	58 (72)	6	1†
1990				
Surgeon	32	10 (31)	0	0
Assistant	59	18 (31)	0	0
1992				
Surgeon	58	7 (12)	0	1
Assistant	47	11 (23)	0	0
1993				
Surgeon	63	16 (25)	0	1
Assistant	68	21 (31)	0	0
1994				
Surgeon	64	10 (16)	0	0
Assistant	69	15 (22)	0	0
Total				
Surgeon	287	84 (29)	5	5‡
Assistant	356	138 (39)	8	1†

\*No patients were prospectively followed between May 1990 and June 1992.

†This patient (Patient 3 in Table 1) had HCV genotype 1 and was considered to have another source of infection.

‡P=0.03.

mechanisms of transmission. The fact that transmission occurred only among patients undergoing procedures performed by the surgeon, not among those undergoing procedures during which he assisted another surgeon, suggests that transmission was associated with percutaneous injuries, most of which occurred during wire closure of the sternum. This procedure has been associated with a high rate of glove perforation,<sup>34</sup> which almost invariably leads to recontact of the surgeon's blood with the patient's open wound.<sup>35</sup> The glove perforation is often not recognized until after the procedure. Other types of percutaneous injuries were less frequent, recognized quickly, and more likely to be followed by safety procedures, such as changing gloves. Hence, although the precise mode of transmission remains unknown, as in many retrospective investigations<sup>30,31</sup> of blood-borne viral transmission from physician to patient,<sup>30,31</sup> wire injury during sternotomy closure is a potential explanation. Such injuries have previously been proposed as a mechanism of surgeon-to-patient transmission of HBV among patients undergoing cardiac surgery.<sup>30</sup> Active liver disease and high levels of viremia in the surgeon may also have played a part.

The average risk of HCV infection after a needle-stick injury involving HCV-infected blood has ranged between 2 and 3 percent,<sup>29</sup> which is between the risk estimates for infection with HBV (30 percent) and HIV (0.3 percent).<sup>31</sup> For HBV and HIV, the risk of transmission from health care worker to patient appears to be substantially lower than the risk with needle sticks, except in special situations. The cases of HCV

infection that we studied resemble reported cases of HBV and HIV infection transmitted in surgical and dental settings, with clusters of cases of infection among patients linked to a single health care worker.<sup>31</sup> Our study was retrospective and included no appropriate controls to permit us to assess risk factors for transmission. In addition, epidemiologic and serologic data from other members of the cardiac-surgery unit were not available.

The observed rate of HCV transmission cannot be used to estimate the average risk of transmission from an infected surgeon to an individual patient during an invasive procedure. Such transmission, however, may not be exceptional. Four additional patients in our prospective studies had postoperative HCV infections that were unrelated to transfusion and remain unexplained. To investigate these cases further and determine the risk of transmission, we have initiated a retrospective study of all former patients of the surgeon and a prospective follow-up study of all patients undergoing cardiac surgery at our institution.<sup>36</sup>

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