

Brief Report

PATIENT-TO-PATIENT TRANSMISSION OF HEPATITIS C VIRUS DURING COLONOSCOPY

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INVASIVE diagnostic or therapeutic procedures may be a route for the transmission of the hepatitis C virus (HCV).¹⁻⁵ In a study of patients in a gastrointestinal-disease unit, endoscopic biopsies were found to be an independent risk factor for HCV infection.⁶ We report the transmission of HCV during colonoscopy from a person known to have HCV infection to two other patients. The patient-to-patient transmission was ascertained by sequencing the nucleotides in the various HCV isolates.

CASE REPORT

A 55-year-old man (Patient 1) and his 54-year-old wife (Patient 2) were referred in October 1995. In June 1995, both had had hepatitis-like illnesses, with nausea, abdominal pain, and conjunctival icterus. They had elevated serum alanine aminotransferase levels and tested positive for HCV antibodies by third-generation assays (Ortho Diagnostic Systems, Roissy en France, France, and Pasteur Diagnostique, Marnes-la-Coquette, France). Both had been regular blood donors for 20 years; they had had normal liver-enzyme levels and negative HCV serologic tests at the time of their most recent blood donations, in January 1995. They had not had blood transfusions, and there was no evidence of intravenous drug use. However, both patients had family histories of colon cancer, and both had undergone colonoscopy on March 16, 1995. Their serum alanine aminotransferase levels one week earlier were normal. Neither patient had previously undergone endoscopy in the same unit.

Liver biopsies were performed in both patients in November 1995, and the histologic activity was measured by the Knodell score.⁷ The biopsies revealed chronic hepatitis, minimal in Patient 2 and moderate in Patient 1 (Knodell scores, 3 and 9, respectively). No fibrosis was found in either biopsy specimen. The results

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of serologic tests for HCV were confirmed by third-generation enzyme immunoassays (Ortho Diagnostic Systems and Abbott Diagnostic Division, Rungis, France). Both patients tested negative for hepatitis B virus (HBV) and human immunodeficiency virus (HIV) types 1 and 2.

METHODS

The temporal relation between HCV positivity and colonoscopy in the two patients led us to suspect a nosocomial infection (Fig. 1). We investigated whether any HCV-positive patients had undergone colonoscopy in the clinic on the same day as our patients and whether any staff member performing endoscopy was HCV-positive. By genotyping and nucleotide sequencing of the HCV genome, we compared the virus isolated from our patients with that isolated from a patient known to be HCV-positive. We studied the procedures used for anesthesia and colonoscopy, in particular the techniques used in cleaning and disinfecting the endoscopes, to try to determine a possible route of infection.

All the staff members involved in performing endoscopy (one gastroenterologist, one anesthetist, one nurse, and one nurse's aide) tested negative for HCV serology by third-generation assays. Only three colonoscopies were performed on March 16, 1995. The first involved a 42-year-old woman (Patient 3) with a history of polypectomy. She was known to have been HCV-positive for one year, but she had not been treated because she had chronic depression. In November 1995, her serum alanine aminotransferase level was 43 IU per liter (normal level, ≤ 40). Serologic tests were positive for HCV and negative for HBV and HIV.

Detection and Titration of HCV RNA

Serum samples from Patients 1 and 2 collected in January 1995 at the time of blood donation and frozen at -80°C and serum samples obtained from Patients 1, 2, and 3 in October and November 1995 were tested for HCV RNA by a reverse-transcription polymerase chain reaction (PCR) with primers from the highly conserved 5' noncoding region of the HCV genome (Table 1). Briefly, RNA was extracted from 100 μl of serum with use of the method described by Chomczynski and Sacchi.⁹ RNA samples were heated for 10 minutes at 70°C and then incubated for 60 minutes at 37°C in a reaction mixture containing reverse-transcription buffer, 10 mM dithiothreitol, 1 mM of each deoxynucleotide triphosphate, 10 U of RNAsin (Life Technologies, Cergy Pontoise, France), 200 U of reverse transcriptase of Moloney murine leukemia virus (Life Technologies), and 0.4 mM outer antisense primer. The samples were then heated to 100°C for 10 minutes. Distilled water, extraction buffer, and HCV-negative serum samples were used as negative controls. Serum positive for HCV RNA served as a positive control. The complementary DNA (cDNA) was amplified by a nested PCR.¹⁰ The first PCR was performed in a 50- μl reaction mixture containing *Taq* polymerase buffer, 1 mM of each deoxynucleotide triphosphate, 1.5 mM magnesium chloride, 1 mM of each outer primer, 1 U of *Taq* polymerase (Life Technologies), and 5 μl of cDNA. There were 35 PCR cycles consisting of denaturation at 95°C for one minute, annealing at 55°C for one minute, and extension at 72°C for one minute.

The second PCR reaction was performed as described above for 25 cycles, with 5 μl of the first PCR product and the inner primers. The PCR products were subjected to electrophoresis in 2 percent agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. The expected size of the amplification product was 190 bp.

HCV RNA was measured in serum by a branched-chain DNA assay (Quantiplex bDNA 2.00, Chiron Diagnostics Europe, Cergy Pontoise, France) in accordance with the instructions of the manufacturer.

HCV Genotyping and Nucleotide Sequencing

HCV genotyping was performed by a line-probe assay according to the instructions of the manufacturer (Innogenetics, Ant-

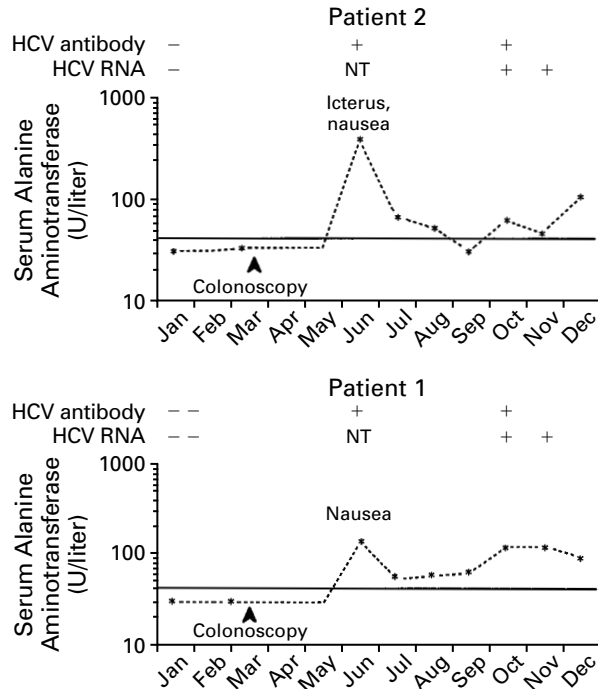


Figure 1. Clinical, Biochemical, Serologic, and Molecular Profiles of Patients 1 and 2.

In these patients, who acquired HCV infection after undergoing colonoscopy, HCV antibodies were detected by third-generation tests and HCV RNA was detected in serum by reverse-transcription PCR, as described in the Methods section. Alanine aminotransferase values are shown on a logarithmic scale, and the horizontal lines indicate the upper limit of the normal range. NT denotes not tested.

TABLE 1. NUCLEOTIDE SEQUENCES AND POSITIONS OF HCV PRIMERS.

PRIMER	SEQUENCE*	NUCLEOTIDE POSITION†
5' Non-coding region		
Outer		
NC1 (sense)	AACTACTGTCTTCACGCAGAA	44-64
NC2 (antisense)	GGTGCACGGTCTACGAGACCTC	311-332
Inner		
NC3 (sense)	CCATGGCGTTAGTATGAGTG	74-93
NC4 (antisense)	GCGACCCAACACTACTCGGCT	243-263
NS3 region		
Outer		
S1 (sense)	CGACTGTAAACACATGTGTCACC	4688-4709
S2 (antisense)	AGCTCGTACCAAGCACAGCC	4902-4921
Inner		
S3 (sense)	GACAGTCGACTTCAGCTTGGA	4712-4732
S4 (antisense)	TCATAGCACTCACACAGGAC	4878-4897

*The nucleotides are listed from 5' to 3'.

†The positions shown are based on those determined by Takamizawa et al.⁸

werp, Belgium). This assay is based on variations in the 5' untranslated regions of the various HCV genotypes. In the line-probe assay, type-specific probes are tagged with poly(T) tails by terminal deoxynucleotidyl transferase and attached to nitrocellulose membranes. Amplified products labeled with biotin are hybridized in reverse to the probes on the nitrocellulose strip. The biotin group is incorporated by using a 5'-biotinylated primer during the amplification. The labeled product obtained from the 5' untranslated region hybridizes with the probe that has a perfect match of sequences. After hybridization, streptavidin labeled with alkaline phosphatase is added and bound to any biotinylated hybrid formed. This technique allows the six major types of HCV and their most common subtypes to be detected.

To study the HCV sequences in the three patients, we performed a nested reverse-transcription PCR in nonstructural region 3 (NS3) with the patients' serum samples (using the primers described in Table 1). The NS3 region probably codes for a protease and helicase. Serum positive for HCV genotype 1b was used as a positive control, and serum negative for HCV RNA was used as a negative control. The NS3 region was chosen for the analysis of sequencing because the 5' noncoding and core regions appear to be quite conserved among the various types of HCV, whereas the putative envelope regions are highly variable even among examples of the same HCV subtype.¹¹

To check the size of the amplification products, the PCR products were subjected to electrophoresis in 2 percent agarose gels and visualized with ethidium bromide staining (expected size, 186 bp). Each sample was tested in triplicate. The PCR products were gently separated from the agarose gel, purified by separation with a minicolumn (Wizard A7170, Promega, Madison, Wis.), and eluted in 50 μ l of distilled water. The total yield of DNA was determined by spectrophotometry; 100 ng of purified DNA product was used in the cycle-sequencing reactions (Prism 401388, Applied Biosystems, Foster City, Calif.), which were performed according to the instructions of the manufacturer. The sequencing products were analyzed with a DNA-sequencing system (Model 373 A, Applied Biosystems). At least two sequencing reactions were performed with each sample, with the appropriate sense and antisense primers (Table 1). The sequences were aligned with use of the Clustal V program, which is widely employed to study the alignment of multiple sequences.¹² This software is marketed under various names by various manufacturers. We used Assemblylign (Kodak International Biotechnologies, New Haven, Conn.). The nucleotide alignments were compared among the three patients, the control serum positive for HCV genotype 1b, and the published sequence of HCV genotype 1b.^{8,13}

RESULTS

Detection and Quantitation of HCV RNA

The serum samples collected from Patients 1 and 2 in January 1995 tested negative for HCV RNA, whereas those obtained in October and November 1995 tested positive. The level of viremia of Patient 3 in November 1995 was 3.5 million genome equivalents per milliliter.

Genotyping and Sequencing of HCV

All three patients were infected with HCV genotype 1b. Nucleotide sequencing of the NS3 region showed that the three patients were all infected with the same isolate, because there was 100 percent nucleotide homology among the three clones. The degree of homology between the nucleotides in the NS3 region, the control serum positive for HCV genotype 1b, and the published sequences of HCV-BK⁸ and HCV-J¹³ genotype 1b isolates ranged from



Figure 2. Nucleotide Sequence of the HCV NS3 Gene Region in the Three Patients Who Underwent Colonoscopy on March 16, 1995, as Compared with the Sequence in Control Serum Positive for HCV Genotype 1b and Two Published Sequences of That Genotype.

There was 100 percent homology among the three patients with respect to the nucleotides sequenced. The dashes in the control and published sequences denote nucleotides identical to those sequenced in the patients; only divergent nucleotides are shown. Underscoring denotes a change in the amino acid sequence expected on the basis of the coding information. Amino acid sequences were not determined. Shading has been added to facilitate the comparison of the sequences.

86.9 percent to 89 percent. The degree of homology in the amino acid sequences deduced from the nucleotide sequences ranged from 89.6 to 96 percent (Fig. 2).

Procedures Used for Colonoscopy, Disinfection, and Anesthesia

Patient 3 underwent colonoscopy from 10:10 to 10:30 a.m., and Patient 2 underwent the procedure from 11:00 to 11:30 a.m. Multiple biopsy specimens were obtained from both patients, because micropolyps were found. Patient 1 had a polypectomy between noon and 12:30 p.m. in which a diathermic loop was used. The same colonoscope (Olympus, Tokyo, Japan) was used throughout all three procedures. The biopsy specimens from Patients 2 and 3 were obtained with the same forceps.

After each procedure, the colonoscope was immediately immersed for 10 minutes in water containing detergent and washed on the outside with disposable swabs. The air, water, and biopsy-suction channels were washed with the same detergent as the colonoscope, with an all-channel irrigator (Olympus). After being rinsed with water, the endoscope and all the internal channels were soaked for five minutes in 2 percent glutaraldehyde. Rinsing in water and drying with compressed air followed.

During the procedures, the biopsy-suction channel was never thoroughly cleaned with an appropriate brush. A consensus report on endoscope disinfection¹⁴ has emphasized that the brush used in cleaning should be appropriate for the instrument and channel size. This mechanical cleaning is used to remove residual tissue, the presence of which may contribute to the failure of the cleaning and disinfection procedures. In interviews, nurses stated that this cleaning step was never performed in the clinic. After each procedure, the biopsy forceps and the diathermic

loop were cleaned mechanically in detergent and in glutaraldehyde, but they were not autoclaved.

The same procedure for anesthesia was used in each patient. An intravenous line was inserted, and anesthesia was induced by injecting fentanyl and midazolam directly into the line. Anesthesia was maintained by injecting propofol continuously into the intravenous line with an electric syringe. The intravenous tubing was changed after each patient's procedure. Because Patient 3 was known to be HCV-positive, the intravenous line and the syringes used to inject the fentanyl, midazolam, and propofol were thrown away after that patient's procedure, which was the first of the three. During the interval between the procedures in Patient 2 and Patient 1, the intravenous lines and the needles were changed, but the same syringes of fentanyl, midazolam, and propofol were used.

DISCUSSION

The timing of the events and the molecular characterization of the various HCV isolates provide evidence that HCV was transmitted during colonoscopy. There are few documented cases of viral transmission during endoscopy.¹⁵ One case of transmission of HBV in this manner has been reported.¹⁶ Acute hepatitis C was reported in a patient 10 weeks after the patient underwent retrograde cholangiography with sphincterotomy; no genotyping or nucleotide sequencing was performed, and HCV could not be proved to have been transmitted during the endoscopy.¹⁷ In both these cases, the disinfection procedure used was considered inadequate.

We suggest that during the disinfection of the colonoscope after the procedures in the patients we describe, two recommendations on endoscopic disinfection made by the American Society for Gastrointestinal Endoscopy¹⁴ and the British Society of Gastroenterology¹⁸ and the working party of the

World Congresses of Gastroenterology¹⁹ were not followed. From our investigation it appeared that the biopsy-suction channel was never cleaned with a brush and that the accessories that breach the mucosa, such as the biopsy forceps and the diathermic loop, were not autoclaved after each use. Recently, the effectiveness of manual cleaning and disinfection in preventing the transmission of HCV was assessed by testing the effluent from the endoscope biopsy channel by reverse-transcription PCR for the presence of HCV RNA.²⁰ That study involved 39 patients with chronic hepatitis C who underwent gastroscopy with biopsy. Sterile water was flushed through the biopsy channel immediately after the removal of the endoscope, after cleaning with a detergent solution, and after a final disinfection with glutaraldehyde. HCV RNA was found in two cases before the cleaning with a detergent, but in every case the water flushed through the biopsy channel after the cleaning step and after the disinfection with glutaraldehyde was negative for HCV RNA.²⁰ These findings confirm that the disinfection procedure is effective when all the recommendations are followed.

Other studies assessing the degree of adherence to guidelines for the cleaning and disinfection of gastrointestinal endoscopes have pointed out the high rate of inadequate disinfection procedures (30 to 100 percent).¹⁴ Failure to follow the recommended procedures can have an important role in the endoscopic transmission of microorganisms.

The possibility that HCV was transmitted because of inadequate procedures in the use of anesthesia should also be considered. We believe this route of transmission is less likely, because the intravenous tubing and all the syringes containing the anesthetic drugs were changed after the first colonoscopy, in which the patient evaluated was known to be HCV-positive. However, inadequate procedures were followed during the other two procedures. Only the intravenous tubing and the needles were changed between the endoscopies of Patients 2 and 1. The anesthesiologist stated that he discarded syringes only after they were used in a patient known to be infected. To justify this approach, he said that he systematically used a check valve to avoid the backflow of blood into the syringe. However, other studies have shown that the rate at which blood contaminates the intravenous tubing used for anesthesia is substantial (0.3 to 3.3 percent of cases).²¹⁻²³ The presence of a check valve and the changing of the needle do not affect the rate at which intravenous lines and syringes become contaminated — a point that should be emphasized, because multidose vials are very commonly used in anesthesia.²¹⁻²³ Moreover, because the assessment of risk factors is not a reliable predictor of which patients have chronic viral infection, the prac-

tice of following strict procedures for anesthesia only in patients with known infections has little rationale and should be abandoned. The standard of care should be to use equipment and ampules only once.

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CORRECTION

Patient-to-Patient Transmission of Hepatitis C Virus during Colonoscopy

Patient-to-Patient Transmission of Hepatitis C Virus during Colonoscopy . On page 237, one of the authors' names is misspelled. "Bertrand Rhin, M.D.," should have been "Bertrand Rihn, M.D."