Hepatitis C virus infection in a hematology ward: evidence for nosocomial transmission and impact on hematologic disease outcome

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Background and Objectives. Hepatitis C virus (HCV) infection is frequent among patients with hematologic malignancies and unapparent routes of infection may be important in this setting. Moreover, the impact of this infection on the outcome of the hematologic disease needs to be better defined.

Design and Methods. To define sources and clinical courses of HCV infection, an epidemiologic study was performed on 13 patients newly admitted over one year who showed transaminase elevation and anti-HCV seroconversion. The investigation, started in August 1998, included laboratory tests and molecular analysis of virus isolates, and was extended to staff and blood donors. Clinical, hematologic and serologic surveillance of all infected patients were part of the subsequent follow-up study which started in September 1998 and was completed in December 2001.

Results. Anti-HCV seroconversion was observed in 13 of 294 patients (4.4%), admitted to the unit from August 1997 and August 1998; 11 of the seroconverted cases had central catheters, 12 received transfusions. Transmission via blood derivatives and staff was ruled out. All patients were infected by genotype 1b and 11 harbored the same viral variant. HCV infection did not influence the course of the underlying disease or the use of specific therapies. Forty months after the outbreak, five patients are alive (one after autologous and one after allogeneic stem cell transplantation), while eight have died, seven of hematologic disease, and one of cardiac failure. None died of liver disease.

Interpretation and Conclusions. The molecular data suggest a patient-to-patient nosocomial HCV transmission. After having analyzed all the possible routes of transmission, a series of preventive measures were adopted: search for HCV RNA in newly admitted patients, protection of mucosae and isolation of patients during neutropenic phases, and avoidance of multidose vials. As regards the impact of HCV infection on the outcome of the hematologic diseases, changes in the scheduled therapy, including stem cell transplantation, were not required. ©2002, Ferrata Storti Foundation

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Hepatitis C virus (HCV) infection is frequent among patients with hematologic malignancies, especially those with lymphoproliferative disorders.[1] In our geographic area we recently found a 16% prevalence of serum anti-HCV among newly diagnosed patients with lymphoma as compared to an 8.5% prevalence in matched hospital controls.[2] This observation corroborates suggestions of a direct role of HCV in the etiology of some of these disorders.[3]

Many hematologic patients, however, acquire HCV infection in the environment in which they received treatment for their disease. The prevalence of anti-HCV in survivors of acute leukemia in the pre-HCV screening era ranged from 10% to 50%.[4–6] After the introduction of routine screening of donated blood for anti-HCV, these figures fell greatly, but a prevalence of 6% to 15% in serum is still reported among both adult and pediatric patients.[7,8] The risk of newly acquired HCV infection is only in part explained by exposure to contaminated blood derivatives.

Patient-to-patient transmission has been documented in such settings as dialysis units,[9-11] hematology wards[12,13] and surgery wards.[14] Patients in hematology units are at particular risk because of high parenteral exposure, immunosuppressive regimens and repeated admissions to hospital. The magnitude of this phenomenon may be underestimated as the clinical manifestations of hepatitis C virus (HCV) infection are modified by immune depression and concomitant therapies, and because seroconversion may occur long after infection or never occur.[15] HCV infection does not influence the course of the underlying disease and the use of specific therapies and it has no impact on medium-term mortality or morbidity.[16-18] Concern, however, arises about the long-term prognosis of HCV infection, as reported in other patient cohorts, such as hemophiliacs.[19]

As part of a surveillance program for HCV infection on all newly admitted patients to our Hematology and Bone Marrow Transplant Unit, in 1997 we introduced screening for anti-HCV in all patients, at entry and at fixed intervals during follow-up, and systematic storage of sera (kept at –80°C). When an outbreak of HCV infection was detected in August 1998, all newly infected patients were enrolled in an epidemiologic investigation aimed at ascertaining the source and the routes of infection; this included retrieval and analysis of stored sera.

The infected patients were followed-up until December 2001, to evaluate the impact of the HCV infection on the course of the underlying hematologic disease, and especially on the feasibility of the scheduled therapies, including autologous and allogeneic stem cell transplantation.

Design and Methods

Design of the study

In August 1998 we observed the first cases of a series of patients showing abrupt elevation of transaminases concomitant with evidence of HCV seroconversion. At this time the surveillance protocol was implemented with clinical, laboratory and epidemiologic studies to clarify the timing and the course of HCV infections, and to identify the source and the routes of transmission. These included review of all nursing procedures, analysis of stored sera (kept at –80°C), recall of all blood donors and molecular analysis of viral isolates. Patients were monitored for clinical and biochemical evidence of liver disease using the following parameters: serial testing of serum alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, albumin and prothrombin time. In September 1998, a follow-up

Table 1. Clinical and serologic characteristics of 13 hematologic patients with HCV infection.

Pt. n.	Diagnosis	Age	Sex	Date of first evidence of	
				HCV-RNA+	Anti-HCV+
1	MDS (RAEB)	70	F	8/98	8/98
2	NHL	51	F	11/97	9/99
3	MM	58	M	5/98	7/98
4	ITP	72	M	7/98	8/98
5	ALL	27	M	2/98	7/98
6	AML	60	F	3/98	6/98
7	AML	28	M	1/98	8/98
8	ALL	27	F	8/97	8/98
9	CML	60	M	8/97	8/98
10	CML-BT	36	M	3/98	4/98
11	AML	54	M	2/98	6/98
12	AML	16	M	5/98	10/98
13	Granular sarcoma	46	M	8/98	8/98

MDS: myelodysplastic syndrome; RAEB: refractory anemia with excess of blasts;
NHL: non-Hodgkin's lymphoma; MM: multiple myeloma;
ITP: idiopathic thrombocytopenic purpura; ALL: acute lymphoblastic leukemia,
AML: acute myeloid leukemia; CML: chronic myeloid leukemia;
CML-BT: chronic myeloid leukemia in blastic transformation.

study of the HCV-infected patients was started, consisting of hematologic (at monthly intervals) and hepatological (every 2 to 4 months) evaluation, which was completed in December 2001.

Patients

Among 294 patients admitted to our Unit in a one-year period, 13 (4.4%) became HCV-RNA positive and were studied for HCV-related infection, time of seroconversion, and its consequences on their underlying hematologic disease. The patients' hematologic diagnoses are shown in Table 1. There were 9 males and 4 females with a median age at entry of 51 years (range 16-72). At first admission no patient had anti-HCV antibodies detectable in serum. Eleven of 13 patients received cytotoxic drugs for a median period of 7 months (range 2-20). Chemotherapy was given by central venous catheters (CVC) managed according to International Guidelines.[20] Multidose vials of heparin solution (5000 U in 100 mL of sterile infusion water) were used for catheter flushing. The multidose vials were prepared and renewed every 12 hours by specialized nurses responsible for multivial compounding and for maintaining administration sets in the ward. Practice techniques were the following: one minute hand washing before any manipulation of vials or CVC, new sterile syringes and needles each time a vial was re-entered, disinfection, prior to use, of the septum of the multidose vials (protected by sterile gauze) with 70% alcohol and disinfection of the

insertion site of the CVC with 10% povidone-iodine. One patient with idiopathic thrombocytopenic purpura (ITP) and one with myelodysplastic syndrome did not receive cytotoxic agents. Twelve patients were transfused with red blood cells or platelet concentrates during periods of pancytopenia; the average number of transfusions was 53.8 per patient (range 10-187). One received intravenous immunoglobulins for ITP.

Informed consent was obtained from all patients and the study design conformed to the guidelines of the 1975 Declaration of Helsinki.

Design and Methods

The inpatient unit accommodates 28 adult patients distributed in 4 rooms with 6 beds and 4 single rooms. Eleven patients were always hospitalized in rooms with multiple beds, and one (with chronic myeloid leukemia, CML) in a single room, while one (with ITP) was an outpatient.

The patients were monitored every 2 to 4 months for clinical and biochemical evidence of liver disease using the following parameters: serial testing of serum alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, albumin and prothrombin time. Serum samples, stored at diagnosis and at every subsequent admission, were kept at -80°C.

Thirty-six patients with chronic HCV infection and 22 with HCV reinfection after liver transplant were used as controls in the analysis of sequence diversity. These two groups of patients were derived from the same hospital over the same period and were studied independently in different investigations.

Anti-HCV, HCV-RNA and HCV genotype detection

Serum samples screened by a 3rd generation HCV ELISA test were confirmed by immunoblotting (Ortho Diagnostic System, Milan, Italy). Serum HCV RNA was tested by nested RT-PCR using conserved primers in the 5'-non-coding region using both commercial (Amplicore, Roche Diagnostic Inc.) and home-made tests with a sensitivity detection limit of 100 copies/mL.[21] HCV genotyping was performed by type-specific primers in the core region according to the method of Okamoto and subsequent modifications.[21]

Cloning and sequencing of the hypervariable region 1 (HVR1)

Sequences from the E2 gene of HCV encompassing the HVR1 region (amino acid 364-422)[22] were amplified by polymerase chain reaction (PCR) following reverse-transcription and cloned in the plasmid vector pCR™II using the TA Cloning Kit (Invitrogen, San Diego, CA, USA). A *nested* protocol was employed for amplification of E2 sequences[23] using the following primers: first round, forward primer: 5'-CGCATGGCATGGGACATGAT-3'; first round, reverse primer: 5'-GG(AG)GTGAA(GA)CAATACAC(TC)GG-3'; second round, forward primer: 5'-ATGCTGGGTACGTGGGCTAAGGT-3', second round, reverse primer: 5'-TTAGGGCGGTCCTGTTGATGTGC-CA-3'.

Sequencing was performed according to Sanger and using fluorescent dideoxy nucleotides on an automated sequencer ABI 310 (Applied Biosystem, Inc, Foster City, CA, USA). A mean of 7.2 clones (range 5-11 clones) were collected and sequenced for each serum sample.

All recommended precautions to prevent PCR *carryover* were taken. PCR reactions used for cloning were performed using highly-processive, proof-reading, thermostable DNA polymerases (Titan RT PCR kit, Boehringer Mannheim, Germany) in order to increase the reaction efficiency and to reduce the incidence of misincorporation artifacts during repeated amplifications.[24]

Sequence analysis

Nucleotide and amino acid sequences encompassing the HVR1 region (amino acids 384-414) were considered for the study of genetic diversity.[22] Sequences were manually aligned and nucleotide or amino acid changes observed at each position between clones within single serum samples or between clones at different times were calculated by pairwise comparison of all sequences. Sequence diversity was defined as the number of differences over the total size of the sequence and expressed as a percentage. Phylogenetic analysis was performed using the PHYLIP program.[25]

For each patient, all sequences were compared and distance matrices were obtained with Kimura's two parameters' model;[26] evolutionary trees were constructed using the neighbor-joining method and they were drawn using the TreeDraw application.

Statistical analysis

All statistical analyses were performed using the SPSS package (SPSS for Windows release 6.0. SPSS Inc. Chicago, IL, USA). The Mann-Whitney U statistics test was applied to the differences in sequence diversity between groups. Statistical significance was considered to exist at $p<0.05$.

Results

Epidemiologic investigation

Among 294 patients admitted to the unit from August 1997 to August 1998 we observed 13 cases of anti-HCV seroconversion (4.4%) (see Table 1 for

a detailed chronology).

Daily records were scrutinized for parenteral exposures. All procedures were reviewed by external referees and staff were interviewed to assess possible violations of infection control procedures. Nursing was found to be consistent with recommended standards and no sporadic violations or systematic errors in safety regulations were found. All staff and all regular blood donors involved with these patients were tested for anti-HCV and serum HCV-RNA. No donor was HCV-RNA positive. Five nurses were anti-HCV positive, and two of them were HCV-RNA positive but they did not harbor the same genotype and HVR1 sequence as the patients.

In September 1998, a series of preventive measures were adopted in accordance with the *Health Management Board* (N.O.): evaluation of HCV-RNA status in all newly admitted patients, protection of exposed mucosal surfaces, isolation in single rooms (when possible) for patients undergoing high-dose induction therapy, and discontinuation of the use of multidose vials. From August 1998 to December 2001, no additional cases of *de novo* HCV infection were observed.

Clinical and laboratory results

All the 13 patients were infected by HCV genotype 1b. HCV-RNA PCR on serum stored at admission was positive in 5/13 cases (#5,6,8,9, and 11). The remainder proved HCV-RNA positive on subsequent sera with a mean latency time from first admission of 6 months (range 2-17). All patients developed anti-HCV positivity: in four (#1, 4, 10, and 13) this was almost synchronous with HCV-RNA seroconversion, in nine it occurred after a median latency of 5 months (range 2-22).

A median of 6.5 months after diagnosis (range 3-24), five patients (#1,3,5,6, and 11; Table 2) experienced overt HCV-related hepatitis with ALT peak levels ranging from 1692 U/L to 4480 U/L, median 1819. Acute hepatitis developed 1-2 months after treatment withdrawal and resolved spontaneously within 6 months (range 1-6) in all cases. In the eight patients not developing acute hepatitis, the ALT peak ranged from 215 to 1457 U/L (median 744). No severe deterioration of liver function was observed thereafter in any of the thirteen patients. HCV infection required no changes in the scheduled regimens which were regularly administered.

Follow-up of patients

As of December 2001, at a median follow-up from detection of HCV-RNA seroconversion of 31 months (range 5 to 52 months), eight patients had died: seven (#1, 2, 5, 6, 7, 8 and 10) of their hematolog-

Table 2. Clinical and serologic follow-up of 13 hematologic patients with HCV infection.

Pt. n.	Diagnosis	AVH	ALT at last follow-up	Outcome of hematologic disease	Survival (mos)*
1	MDS (RAEB)	yes	–	PD	13[†]
2	NHL	no	–	PD after auto-BMT	31[†]
3	MM	yes	–	PR	37[†]
4	ITP	no	89	CR	41
5	ALL	yes	–	PD	14[†]
6	AML	yes	–	PD after auto-BMT	30[†]
7	AML	no	–	PD	14[†]
8	ALL	no	–	PD	16[†]
9	CML	no	44	CR	52
10	CML-BT	no	–	PD	5[†]
11	AML	yes	56	CR	46
12	AML	no	14	CR (auto-BMT)	43
13	Gran.Sarcoma	no	27	II CR (allo-BMT)	40

*AVH: acute viral hepatitis; PD: progressive disease; PR: partial remission; CR: complete remission; auto-BMT: autologous bone-marrow transplantation; allo-BMT: allogeneic bone marrow transplantation; *months from HCV-RNA seroconversion; †:dead.*

ic disease, and one (#3) from cardiac failure. Details of the hematologic and liver disease outcomes 40 months from the outbreak are shown in Table 2. Overall, three of thirteen patients lost serum HCV-RNA, two spontaneously (#3 and 6), one (#9) after interferon treatment for chronic myelogenous leukemia (CML). In this patient, interferon was well tolerated and effective for both diseases, as the outcome of CML was also favorable, with achievement of a complete hematologic remission. The patients achieved a complete cytogenetic response in November 2001, after 6 months of treatment with imatinib mesylate, which was well tolerated and non-toxic for liver function.

Molecular analysis of HVR1 sequences

Molecular analysis of individual HCV isolates in the HVR1 region was performed on follow-up sera of all patients. Unfortunately, most of the sera stored at diagnosis were no longer available as they had been used for HCV-RNA testing. Complete sequential testing, including sera collected at first admission, was possible in only 2 of 13 cases for whom several follow-up samples were available. Intra-sample sequence diversity was first calculated by comparing the sequences derived from any single serum sample. This was significantly lower than that in 2 control groups of 36 immunocompetent subjects with chronic HCV infection and in 22 patients with HCV reinfection after liver transplant (Table 3). Sequence variation over time was calcu-

Table 3. Intrasample nucleotide and amino acid diversity of HVR1 sequences in the 13 hematologic patients included in the study, in 36 immunocompetent patients with chronic hepatitis C and in 22 patients with HCV recurrence after liver transplant.

	1. Hematologic patients	2. Chronic hepatitis patients	3. Liver transplant patients	p* 1 vs 2	p* 1 vs 3
Nucleotide diversity	0.008±0.01	8.892±8.186	0.039±0.062	<0.0001	0.006
Amino acid diversity	1.399±1.871	25.99±16.53	5.846±8.493	<0.0001	0.0063

*Mann-Whitney U test.

Table 4. Intersample nucleotide diversity of HVR1 sequences in 2 hematologic patients and in control patients over comparable periods.

Patients	Nucleotide diversity
N. 11	0.081
N. 12	0.006
Chronic hepatitis*	7.24±7.35
HCV asymptomatic carriers#	13.19±9.07
Liver transplant patients°	0.04±0.07

*Mean of 26 patients, over one year (21); #mean of 10 patients, over one year (21); °mean of 22 patients, over two years (Silini, unpublished data).

lated for the 2 subjects whose serial sera were available. Inter-sample sequence diversity values were much lower than those in immunocompetent subjects over comparable periods, whereas our values were similar to those observed in immunodepressed liver transplant patients (Table 4).

The HVR1 sequences derived from the different patients were then compared. High homology, both at nucleotide and amino acid level, was shown by direct comparison of the aligned sequences (Figure 1), and also expressed by the low values of interpatient diversity compared with the diversity between viral isolates from 10 unrelated subjects infected by the same 1b genotype (mean nucleotide diversity 0.139 vs 0.739; mean amino acid diversity 0.185 vs 0.568). Eleven of 13 sequences were essentially identical, whereas two isolates from patients #4 and #9 were clearly different. This conclusion was reinforced by the analysis of the phylogenetic relations between isolates (Figure 2A) which showed two segregation groups, one including the 11 identical sequences of our cohort and a second more heterogeneous group encompassing 10 unrelated HCV 1b
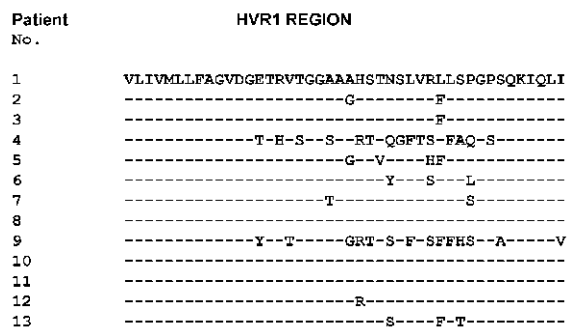

Figure 1. Aligned HVR1 amino acid sequences of the 13 hematologic patients included in the study. Only the most represented sequence for each patient is shown.

isolates and the two isolates from patients #4 and #9. One of these patients, carrying a CVC for infusion of chemotherapy, was always accommodated in a single room, the other was an outpatient.

In order to reconstruct possible transmission routes in our cohort, we applied bootstrap analysis to the phylogenetic tree to detect subgroups of homology. No consistent segregation of sequences was observed, as is shown by the low reproducibility levels of the phylogenetic relationships between isolates (Figure 2B). Furthermore, no subgroup was correlated with the time of admission, or with proximity between patients, except the sequence from patient 8 which reproducibly segregated from the others. Interestingly, this patient was the first involved in the outbreak.

Discussion

Since the introduction of routine screening of blood for anti-HCV and the steep decrease in the incidence of post-transfusional hepatitis,[27] patient-to-patient transmission has become the commonest mode of nosocomial HCV infection. Data on anti-HCV seroprevalence in survivors from hematological malignancies, both adults and children, suggest that these infections are more frequent than was previously thought.[5-7] Only two studies give detailed epidemiologic evidence of hepatitis C transmission in hematology-oncology units.[9,13]

Here we describe a third nosocomial outbreak of HCV infection which we documented at epidemiologic, clinical and virological levels taking advantage of a prospective surveillance program started in 1997 calling for systematic sera storage and follow-up of patients. De novo HCV infection was observed in 13 (4.4%) of 294 newly admitted
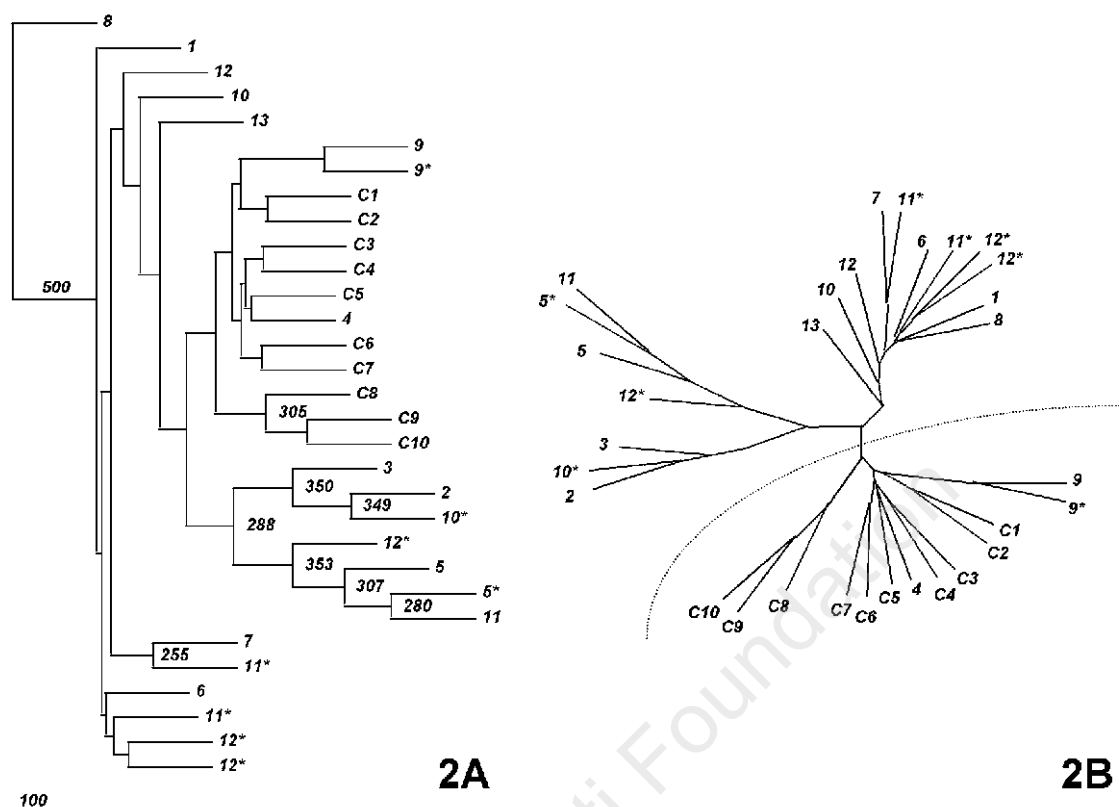
Figure 2. A) Phylogenetic tree (phylogram) of the nucleotide sequences of the 13 hematologic patients and 10 unrelated controls with HCV type 1b infection. Only bootstrap values considered significant are reported (analysis performed on 500 replicates). B) Phylogenetic tree (unrooted) of the nucleotide sequences of the 13 hematologic patients and 10 unrelated controls with HCV type 1b infection. Segregation into two distinct groups is appreciable. *C (controls); *(multiple sequences of same patient).*

patients examined over a one-year period (August 1997-August 1998). This figure is close to the prevalence of anti-HCV observed in adult patients surviving chemotherapy for leukemia and lymphoma in the same area, in whom anti-HCV positivity rates of 6.53% in 1997 and of 4.98% in 1998 were reported, independently of the development of an overt HCV infection.[2]

Sequence analysis of viral RNA extracted from the patients' sera established that a unique viral variant circulated among 11 of these 13 patients. As transmission through blood derivatives or by staff was excluded, this evidence is consistent with patient-to-patient transmission. This single viral variant propagated virtually unchallenged over 22 months as demonstrated by the lack of sequence selection in the HVR1 region both within and among subjects,[28] at variance with other known patient categories. It

was possible to calculate sequence variation over time for 2 subjects for whom sera at entry were available for molecular study. As expected, this was significantly lower than in 2 control groups of immunocompetent subjects, whereas it was similar to that observed in immunodepressed subjects,[15,28] as also documented in a group of liver transplant patients from the same institution. Our experience also evidences a high inter-patient homology, both at nucleotide and amino acid level. This observation best testifies to the profound immune depression, caused both by the underlying diseases and by the pharmacologic treatment characteristically administered to hematology-oncology patients and central to all manifestations of HCV infections in this setting.[15] Whether this variant was specifically adapted or emerged by pure chance is debatable. It is remarkable, however, and suggestive of a particularly

fit strain, that even previously HCV-infected patients - five patients were already HCV-RNA positive at admission - also became infected by this HCV variant, as some time after admission they all harbored HCV sequences identical to those of newly infected patients. Superinfection could be taken into account as previously reported in this[12] and other clinical settings.[10,29] Although we found no direct evidence for it, as only a few sera at entry were available for molecular analysis, this is nonetheless the most likely explanation of our observations, as the patients were all new to the unit when first admitted and they had been referred from different sources.

The origin of the outbreak was not identified, though circumstantial evidence suggested that patient #8 was the index case because she was the first found to be infected and she was HCV RNA positive soon after admission. We could not identify a definite route of transmission for these infections, nor establish connections between infected patients by molecular biology or by classical epidemiologic approaches. No patients were ever present in the unit simultaneously, but treatment periods overlapped. Both males and females were involved, although the 2 groups were treated in separate areas and so had limited contact except for taking meals together.

It is unlikely that a single cause, such as a systematic error in safety protocols or contamination of instruments, could explain all the infections, since the extensive review of medical and nursing procedures always found them complying with recommended standards. There have, however, been several reports of viral and bacterial infections linked to contaminated multidose vials.[13,30,31] The practices more at risk included reusing syringes for more than one patient, and never or rarely disinfecting the septum of multidose vials prior to use.[30] Contamination of multidose vials seems unlikely in the present case, given the strict hygienic rules adopted at our Unit in managing these medications (*see Design and Methods*). Furthermore, in outbreaks due to contamination of a multidose vial, infections do not usually occur over a prolonged period of time, as they did in our series. It is also interesting to observe that one patient (#9), receiving chemotherapy by CVC and submitted to the same practice of catheter flushing using heparin solution from a multidose vial, but always accommodated in a single room, developed an HCV infection with a different viral sequence. Alternatively, unapparent routes of transmission, probably involving the passage of minimal quantities of blood or biological fluids, should be considered.[9,12] Infection by aerosol during the phase

of aregenerative mucosal lesions has been suggested as an alternative mode of transmission,[12] although it has not been proven. Lodging patients in rooms with multiple beds might magnify this risk. Indeed, close contact of patients sharing rooms offers many opportunities for transmission mediated by staff or visitors, and by the patients themselves.[9] Because of the continuous turnover of patients and the many, repeated occasions of contact with infected subjects, it was impossible to obtain more than circumstantial evidence for a role of shared environments in HCV infection transmission. Interestingly, however, the only two patients of the group with different viral variants were an outpatient and a patient always accommodated in a single room.

The network of causation for HCV infection in this high-risk setting may be too complex to clarify, but this could be irrelevant from a practical standpoint. In our experience, as in previous studies,[12,13] increased awareness of the problem and the adoption of measures, such as protection of mucosal surfaces, discontinuation of the use of multidose vial medications and isolation of patients, terminated the chain of infection almost immediately. Although we cannot generically blame the environment to explain or excuse these infections (a preventable passage of biological material must have occurred), it is clear that even units complying with the currently accepted safety standards are far from being immune from risk. Precautions regarding staff, invasive procedures and treatment modalities are maximal but a specific design of overall assistance and better accommodation in hospitals are also needed.

Immunosuppression influenced the clinical manifestations of HCV infection in these patients.[15] Primary infection was entirely silent as it probably occurred during the first period of induction therapy when mild liver dysfunction is common.[18] During follow-up, we saw transient ALT elevation, usually in the post-treatment immune recovery phase. Anti-HCV seroconversion was not concomitant with the clinical manifestations and occurred up to the 17th month after admission, as also documented in pediatric patients.[7,32] The only reliable way to detect HCV infection was to test routinely for HCV RNA at entry and at fixed intervals during hospitalization and for anti-HCV during follow-up to detect late seroconversion.

In conclusion, taking into account all the possible routes of transmission,[12,13,30] we adopted specific measures in order to prevent HCV infection in our hematology wards: repeated controls of strict observance of nursing procedures; testing for HCV RNA by

RT-PCR at first admission of the patient and at fixed intervals thereafter, as conversion times are often delayed; protection of mucosal surfaces and isolation of patients during the neutropenic phases; avoidance of medications from multidose vials. After all these measures had been implemented, from August 1998 to December 2001 no additional cases of *de novo* HCV infections were observed. Although these preventive measures proved to be successful in terminating the chain of infections, no specific cause-effect relationship for any of the measures adopted was established.

As regards the impact of HCV infection on the outcome of the hematologic disease, after a follow-up period of forty months we can confirm recent reports[33-35] that HCV infection does not significantly influence the course of the underlying disease or the ability to undergo specific treatments, including stem cell transplantation. More prolonged observation is, however, warranted to evaluate the long-term effects of HCV infection in this category of patients, as they may be a matter of concern, especially in children whose life and cure expectancies are higher.[8]

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Disclosures

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PEER REVIEW OUTCOMES

Manuscript processing

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What is already known on this topic

Hepatitis C virus (HCV) is highly prevalent among patients treated in hematology wards. Although HCV is involved in the pathogenesis of some malignancies of the B-cell lineage, several lines of evidence indicate that hematologic patients may acquire their HCV infection in the environment in which they are treated.

What this study adds

This study describes the propagation of primary HCV infection among 13 (4.4%) of 294 patients treated in a Haematology and Bone Marrow Transplant Unit, over a 22-month period. The transfusional origin of the cluster route was excluded, and molecular analysis showed that a single HCV variant was implicated in 11 of the 13 cases, suggesting patient-to-patient transmission.

Potential implications for clinical practice

The results of this survey support the need to adopt stringent hygienic measures to prevent nosocomial spread of HCV. In particular, the use of multidose vials must be abandoned.

Paolo Rebulla, Associate Editor (Milan, Italy)