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Detection of hepatitis C virus in an exhumed body identified the origin of a nosocomial transmission that caused multiple fatal diseases

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SUMMARY

Background: Medico-legal conflicts arise when it is difficult to prove the cause of nosocomial infections.

Aim: To report an outbreak of patient-to-patient transmission of hepatitis C virus (HCV) through the repeated use of a multi-dose saline flask during the rinsing of central venous catheters.

Methods: Blood samples were taken from each patient for the comparative analysis of their HCV RNA strains. No samples were available for one patient who died before the investigation started. Despite the known lability of HCV RNA, the body was exhumed four months after burial and postmortem samples were collected. HCV RNA was extracted successfully from liver and spleen samples. Genotyping of all the HCV strains was performed by sequence analysis of the 5′NC untranslated region, the E1 core conserved region and the E1/E2 hypervariable region.

Findings: Forensic investigators retraced the route used by two ward nurses, when saline catheter flushes were given to 14 patients with each nurse administering to seven patients. The comparative phylogenetic analysis of all case strains identified the deceased patient as the source of contamination to five patients.

Conclusions: This study highlights the value of sequence analysis as a tool for solving medico-legal conflicts. The High Court of Justice found that a health worker's re-use of a contaminated needle resulted in the nosocomial transmission of HCV.

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Introduction

Hepatitis C virus (HCV) transmission in healthcare settings is a recognized public health problem, even in developed countries [1]. Unsafe, preventable and disconcerting practices have been reported from re-use of syringes and needles during drug dilution or reconstitution and/or errors during the manipulation of connector lines in patients who received radiopharmaceuticals to study myocardial perfusion [2], and in subjects who underwent contrast-enhanced computed tomography scanning [3].

Acute hepatitis C infection has a subclinical or entirely asymptomatic course in most affected people. The disease resolves spontaneously in a minority of patients, and chronic infection develops in approximately 80% of cases with a subsequent risk of liver-related complications [4]. A newly acquired HCV infection may go undetected, even in a healthcare setting. Forns *et al*. [5] reported an overall rate of 0.46% for positive HCV serology tested six months after hospital discharge, with the rate increasing to 1.7% in patients with a hospital stay exceeding 10 days. Few data are available on the outcome in patients with nosocomial transmission and severe comorbidities; in a cohort of 64 oncology patients followed for six years, severe liver disease occurred in eight patients, one patient underwent liver transplantation and three patients died due to liver-related disease [6]. More than one possible mechanism of transmission may occur in each patient, making it difficult to identify the index case at an early stage and subsequently perform an appropriate clinical investigation including all exposed patients in a short interval of time. Source identification employs epidemiological and laboratory analysis, as well as molecular tools.

This paper reports the field and virological investigation of six cases of acute hepatitis C diagnosed in haematology and internal medicine units. Genomic HCV RNA was obtained from the postmortem liver and spleen of the suspected index case because no stored biological samples were available. To the authors' knowledge, this is the first time that HCV RNA has been detected in an exhumed corpse four months after burial. Due to this result, it was possible to identify the index case and, subsequently, the nosocomial transmission of HCV, leading to the definite identification of unsafe practices and to a strong recall of recognized guidelines.

In response to this outbreak, an epidemiological investigation was conducted by a legal team instructed by a judge. This paper reports the epidemiological investigation undertaken to confirm the presence of the outbreak; to identify the source(s) of infection; to elucidate the transmission pathway(s); and to define any responsibilities or misconduct, the clinical outcome of infected patients and any changes in life expectancy. The report was written after completion of the criminal proceedings, and was written in accordance with 'The ORION statement: guidelines for transparent reporting of outbreak reports and intervention studies of nosocomial infection' [7].

Materials and methods

Patients

On 16th January 2006, a male patient (P1) was admitted to the haematology unit of an Italian hospital with previously diagnosed acute myeloid leukaemia. He later developed acute hepatitis C, although he had no known risk factors for infection. A few days later, a second diagnosis of acute hepatitis C was made in the same ward for another patient (P2) with the same admission diagnosis and no reported suggestive clinical HCV history. The third case of acute hepatitis C occurred in a patient (P3) with multiple myeloma. Fresh samples were collected at the time of diagnosis (P1 and P2, 6th February 2006; P3, 14th February 2006). All three patients had been hospitalized in the same ward in previous months. None of the patients had behavioural risk factors for HCV infection. A review of all known HCV-positive patients who had been hospitalized in the haematology unit and adjoining internal medicine unit was undertaken, as well as a sero-virological survey on all inpatients from October 2005 to April 2006. Both units were located in the east wing of the hospital and shared nursing personnel and healthcare services. The review led to the identification of P4, a HCV-positive female with pneumonitis, who had been hospitalized in the internal medicine unit from 21st November to 19th December 2005. Further investigation identified a second HCV-positive patient (P5), who was admitted with bronchopneumonia on 26th November 2005 and died on 29th December 2005. The sero-virological survey identified three other patients (P6, P7 and P8) who were diagnosed with acute HCV infection. Fresh or stored serum samples were collected from all patients in March and April 2006, with the exception of P5 who had died four months previously and for whom no stored serum samples were available. The body was exhumed and found to be well preserved with minimal signs of decomposition, probably due to the fact that it had been buried in the earth and there had been constant low temperatures throughout the winter period. Liver and spleen samples were taken but no blood was collected as it was totally haemolysed.

Epidemiological investigation

The medical records of the first three patients with acute HCV infection were reviewed, and data on risk factors, diagnostic procedures, surgical interventions, medications and other treatments were collected. Detailed interviews were held with medical and nursing staff with special attention to compliance with infection control practices. A review was carried out on all HCV-positive patients admitted to the haematology unit or internal medicine unit between October 2005 and April 2006; both units were located in the east wing of the hospital and shared nursing personnel and healthcare services. All patients who were admitted to one of the two units in the same period were tested, as well as all medical and nursing staff. All death certificates issued between October 2005 and April 2006 were checked for diagnoses of acute hepatitis. All donors of blood components transfused to the patients who experienced an acute HCV infection were recalled and retested for HCV.

Virological investigation

HCV antibody screening tests were conducted using fresh or stored serum samples, and the Ortho HCV Version 3.0 ELISA Test System (Ortho Clinical Diagnostics, Raritan, NJ, USA). Confirmatory testing was performed using the recombinant immunoblot antibody assay (Chiron RIBA HCV Test, Version 2.0; Roche, Basel, Switzerland). The presence of HCV RNA was

analysed in anti-HCV-positive serum samples from all patients with the exception of P5, for whom only liver and spleen biopsy samples were available. Genomic RNA was extracted from all sample types using a commercial kit (QIAamp Viral RNA kit; Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. As the kit had not been validated for postmortem samples, an internal control was included to assess the efficacy of RNA extraction. HCV RNA was then amplified using a standardized, automated, qualitative reverse transcription polymerase chain reaction assay (COBAS AMPLICOR HCV Monitor, Version 2.0; Roche), as recommended by the manufacturer. The detection limit was 50 IU/mL. Quantitation of serum HCV RNA was determined using the Versant HCV bDNA 3.0 assay (Bayer HealthCare, Diagnostics Division, Tarrytown, NY, USA).

HCV genotypes were determined using a line-probe hybridization assay (Inno-LiPA HCV; Innogenetics, Gent, Belgium). In order to confirm the HCV source and nosocomial transmission, HCV RNA-positive samples from all patients (P1, P2, P3, P4, P5, P6, P7 and P8) were re-amplified and sequenced for the highly conserved 5′ untranslated region (5′ UTR) and the E1/E2 hypervariable region. Multiple sequence alignments and phylogenetic analysis were performed using ClustalX and Phylip programs, respectively [8,9].

Results

Patient characteristics

In total, eight patients with HCV (six with acute HCV and two with a chronic form) were involved in the nosocomial outbreak. Their main clinical and virological characteristics are described in Table I.

Epidemiological results

All healthcare workers were tested and were anti-HCV negative. The possible role of transfusion was excluded because no common donor to the first three patients who developed acute hepatitis (P1, P2 and P3) was identified, and HCV screening was confirmed negative. A single blood component transfusion was made to P1. P1, P2 and P3 were hospitalized on the same ward and all three had indwelling central venous catheters (CVCs) from 11th November 2005 to 14th December 2005. On the basis of this finding, all living patients admitted from 29th November 2005 to 14th December 2005 with a history of indwelling CVCs were recalled. In the event of death (six patients), clinical reports were examined to look for a diagnosis of acute hepatitis; no HCV infections were reported. P1 underwent a surgical intervention, a laryngoscopy was performed in P2 and no invasive procedure was reported for P3. All eight patients were only present at the same time for four days, from 12th December 2005 to 15th December 2005, suggesting that a unique event was likely to be responsible for the nosocomial transmission of HCV. Seven of the eight patients had CVCs inserted at some point during their hospitalization, but only six patients (P1, P2, P3, P5, P6 and P7) had indwelling CVCs in the same 48-h period. Furthermore, verification of the CVC insertion and removal times for each patient evidenced that they all had a CVC from 6:00 pm on 13th December 2005 to 5:00 pm on 14th December 2005. On this basis, the only two patients (P4 and P8) who did not have indwelling CVCs during the crucial 23-h period were excluded from the investigation, while P5 (diagnosed previously with chronic HCV infection) was considered to be the most likely source of the HCV transmission. The only shared event involving all patients, which could be accountable for the transmission of HCV, was the saline flush after blood withdrawal from the CVCs. Interviews with the nursing staff and checks of the working schedule and laboratory barcode data, which identified patient and blood sample collection times, showed that the transmission event could only have taken place on 14th December 2005 between 7:00 am and 7:30 am, reducing the crucial period to 30 min. CVC lines were routinely flushed using a multi-dose 250-mL flask and a single syringe for each action on the patient. The only exposure that yielded a trend for an association with acute HCV infection was the re-use of a disposable needle used for saline flushing of the CVCs after blood collection. On 14th December 2005, two nurses administered to 14 patients in the internal medicine unit and adjoining haematology unit. Each nurse administered to seven patients, and blood was collected from the CVCs followed by saline flushing using the same 250-mL multi-dose flask of saline solution. At the end of the ward round, two containers, each with seven blood collection tubes, were handled. One container was later found to have seven HCV RNA-negative blood samples, while six of the seven tubes

Table I
Main clinical characteristics of the case patients

Patient	Age at outbreak (years)	Sex	Main comorbidities	Hepatitis diagnosis	HCV RNA at diagnosis (copies/mL)	Hospital recovery	Death
P1	38	M	AML	Acute	>40,000,000	26 November–27 December 2005	19 February 2008
P2	67	M	AML	Acute	>40,000,000	11 November–16 December 2005	8 February 2006
P3	37	M	MM	Acute	9,953,907	29 November–12 December 2005	10 April 2007
P4	86	F	Multiple[a]	Chronic	n.d.	21 November–19 December 2005	n.d.
P5	86	F	Bronco-pneumonia	Chronic	n.d.	26 November–29 December 2005	29 December 2005
P6	84	F	Cerebral diseases, stroke, diabetes	Acute	330,988	8–27 December 2005	n.d.
P7	85	F	Stroke, hypertension	Acute	<3200	7–15 December 2005	n.d.
P8	83	F	Cerebral atrophy	Acute	<3200	12–16 December 2005	n.d.

M, male; F, female; AML, acute myeloid leukaemia; MM, multiple myeloma; n.d., no data.
[a] Parkinson's disease, cerebral diseases, stroke, chronic kidney failure, hypertension and pneumonitis.

in the other container were HCV RNA positive and one was HCV RNA negative. The forensic investigators retraced the route taken by the two nurses on the morning of 14th December 2005 and deduced that, theoretically, the only possible mechanism for the transmission was that one nurse's first administration was to an uninfected patient and the second administration was to P5, the source of HCV contamination, followed by the five infected patients (P1, P2, P3, P6 and P7) who subsequently acquired acute HCV infection due to re-use of the contaminated needle. The other nurse had administered to seven uninfected patients. This contamination event did not include P4 and P8, who were hospitalized in the adjoining west wing, confirming their previous exclusion from the case based on the absence of CVCs on the day in question. The procedures to manage a venous line were reviewed and changed; only single-dose vials should be used for washing catheters.

Virological results

HCV antibodies were detected in six patients: two chronic HCV patients (P4 and P5) and four of the six acute HCV patients (P3, P6, P7 and P8). Data from the look-back study showed that five of the six acute HCV patients (P1, P2, P3, P6 and P8) were HCV seronegative on 13th December 2005 (candidate date for nosocomial HCV infection). Seroconversion was subsequently identified in three patients (P3, P6 and P8), while P1 and P2 remained seronegative. Serology data was only registered in one instance for P7, who was identified as seropositive in May 2006. Regarding the two chronic HCV cases, P4 was HCV seropositive in a sample taken on 22nd November 2005. During hospitalization, all laboratory tests for P5 were dedicated to support the treatment management for severe cardiovascular symptoms. Thus, serological tests for HCV antibodies were not performed as this patient had a positive medical history of chronic HCV-related hepatitis, confirmed by elevated liver enzymes. However, specific HCV antibodies were detected in the exhumed corpse four months after death. HCV RNA was detected in fresh, stored samples from six patients (P1, P2, P3, P6, P7 and P8) and in biopsy samples recovered from the exhumed corpse of P5.

Sequence alignments of the HCV 5′ UTR from all patients revealed that all strains were genotype 2a with 100% homology to the reference 2a strain, HCJ5 (data not shown). Phylogenetic analysis documented that the homology of the patients' strains differed from 25 non-case HCV strains from Italian patients in the same geographical area (data not shown). The second sequence analysis was of the hypervariable E1 core region. This analysis placed five patients (P1, P2, P3, P6 and P7) in a cluster of very similar but not identical HCV strains, which was separate from nine non-case strains from nine patients in the same geographical area. Two HCV strains from P4 (chronic HCV infection) and P8 (acute HCV infection) remained separate and distinct from the other case HCV strains, indicative of a different origin of infection (Figure 1). The HCV strain from P5 was not sequenced due to a lack of sufficient sample. The third phylogenetic analysis was of the hypervariable E1/E2 region, which was sequenced in samples from five patients (P1, P2, P3, P5 and P6). The comparative analysis with 25 non-case HCV strains from subjects from the same geographical area placed the five case strains in a cluster of very similar but not identical HCV. One sample from P4 was clearly separate from the first cluster (Figure 2). The HCV strains from P7 and P8 were not sequenced due to a lack of sufficient sample. The sequence analysis of all HCV strains documented that P5 was the most probable source of transmission of HCV infection to five patients (P1, P2, P3, P6 and P7).

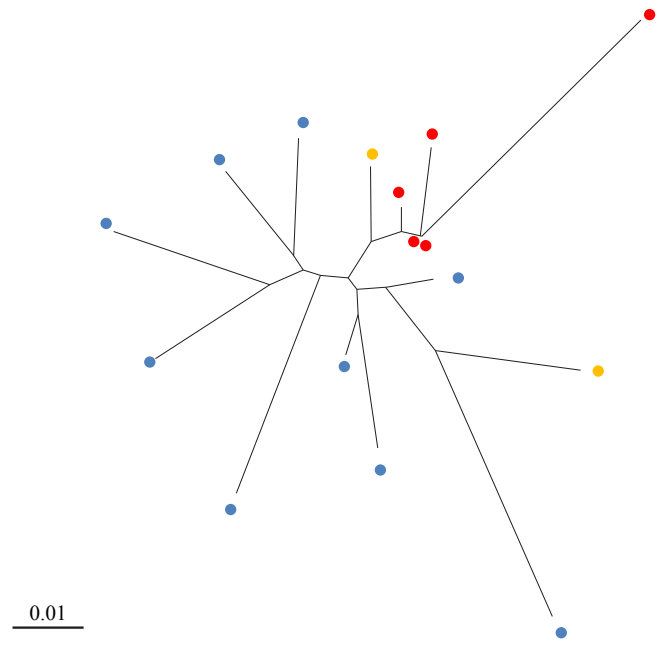

Figure 1. Neighbour-joining unrooted phylogenetic tree of the hepatitis C virus core protein gene. Red circles, haematology unit patients; yellow circles, internal medicine unit patients; blue circles, non-case patients. Line bar indicates nucleotide substitutions per site.

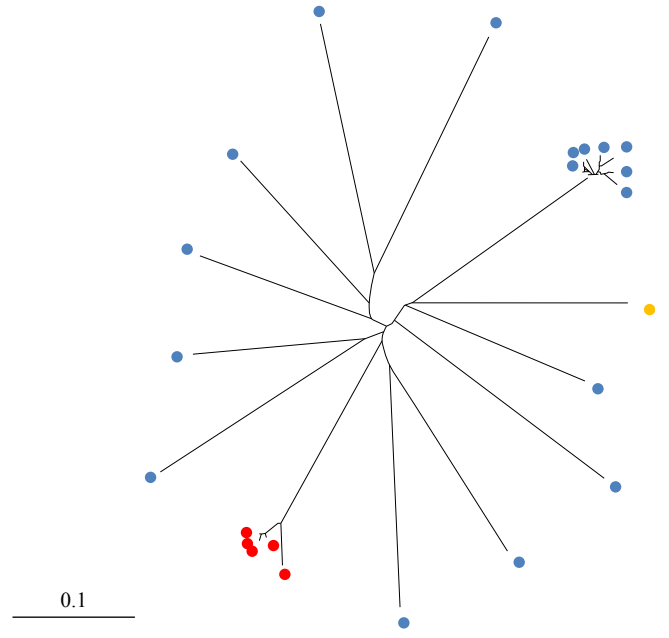

Figure 2. Neighbour-joining unrooted phylogenetic tree of the hepatitis C virus polyprotein E1/E2 region. Red circles, haematology unit patients; yellow circle, internal medicine unit patient; blue circles, non-case patients. Line bar indicates nucleotide substitutions per site.

Discussion

The description of this cohort of HCV-infected patients was only possible once outbreak-associated lawsuits had been completed. In January 2006, five patients (P1, P2, P3, P6 and P7) acquired an acute HCV genotype 2a infection in two adjoining wards: the haematology unit and the internal medicine unit. This case series describes an outbreak of acute HCV infection which initially involved three haematology patients (P1, P2 and P3): one death occurred during the early phase of viral disease (P2, acute myeloid leukaemia) and two deaths occurred during the chronic evolution of the viral disease (24 and 14 months after diagnosis, respectively). HCV disease can be a major concern for short- and long-term outcome of patients with haematological malignancies; death due to acute infection [10] and a high rate of acute exacerbation of chronic infection (occurring in 73% of studied patients, $P<0.0001$) [11] have been described. P1 and P2 did not develop specific antibodies; this is not an unexpected finding in severely immunosuppressed patients [12], and the diagnosis of acute infection was established taking into account initial plasma HCV RNA viral loads and the results of molecular epidemiology. A 13% rate of absence of antibodies was reported in a cohort of patients with lymphoproliferative diseases [13], but their viral loads ranged from 15 to 1170 IU/mL; in fact, P1, P2 and P3 had very high plasma values at initial evaluation (>400,000,000 copies/mL for P1 and P2, 9953,907 copies/mL for P3).

To the authors' knowledge, this is the first report of identification of HCV in specimens collected from an exhumed cadaver approximately four months after death. This exceptional event was possible as the body was well preserved with minimal signs of decomposition even after burial, probably due to the constant low temperatures throughout the winter period. For this reason, the expected decomposition processes had not started. In fact, it is well known that RNA is easily degradable by numerous chemical and physical agents, unlike DNA viruses. Therefore, it was considered probable that the quality and quantity of RNA would be compromised due to degradation and/or the presence of high concentrations of anaerobic and aerobic microbes. Instead, due to the favourable condition of the internal organs recovered during the postmortem, amplifiable RNA was obtained.

Sequence analysis of the strains isolated from the eight case patients identified six patients with homologous HCV RNAs, suggesting a common source of infection, and two patients with genetically unrelated strains, suggesting that these individuals were not involved in the outbreak under consideration. Of the six patients with the closely related viral strain, five developed acute HCV infection while one patient (P5) was known to have chronic hepatic disease. Therefore, P5 was the only possible source of the infection.

These results demonstrate that the origin of the HCV strain obtained from postmortem samples from P5 allowed identification of nosocomial transmission of HCV infection from the index case to five other patients in the same ward due to inadvertent contamination of saline solution with HCV-infected blood.

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Conflict of interest statement
None declared.

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References

[1] Shemer-Avni Y, Cohen M, Keren-Naus A, Sikuler E, Hanuka N, Yaari A, et al. Iatrogenic transmission of hepatitis C virus (HCV) by an anesthesiologist: comparative molecular analysis of the HCV-E1 and HCV-E2 hypervariable regions. Clin Infect Dis 2007;45:e32—8.
[2] Patel PR, Larson AK, Castel AD, Ganova-Raeva LM, Myers RA, Roup BJ, et al. Hepatitis C virus infections from a contaminated radiopharmaceutical used in myocardial perfusion studies. JAMA 2006;296:2005—11.
[3] Quer J, Esteban JI, Sanchez JM, Otero T, Rius C, Coll M, et al. Nosocomial transmission of hepatitis C virus during contrast-enhanced computed tomography scanning. Eur J Gastroenterol Hepatol 2008;20:73—8.
[4] Grant WC, Jhaveri RR, McHutchison JG, Schulman K, Kauf TL. Trends in health care resource use for hepatitis C virus infection in the United States. Hepatology 2005;42:1406—13.
[5] Forns X, Martinez-Bauer E, Feliu A, Garcia-Retortillo M, Martin M, Gay E, et al. Nosocomial transmission of HCV in the liver unit of a tertiary care center. Hepatology 2005;41:115—22.
[6] Mailliard ME, Capadano ME, Hrnicek MJ, Gilroy RK, Gulizia JM. Outcomes of a patient-to-patient outbreak of genotype 3a hepatitis C. Hepatology 2009;50:361—8.
[7] Stone SP, Cooper BS, Kibbler CC, Cookson BD, Roberts JA, Medley GF, et al. The ORION statement: guidelines for transparent reporting of outbreak reports and intervention studies of nosocomial infection. Lancet Infect Dis 2007;7:282—8.
[8] Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997;25:4876—82.
[9] Felsenstein J. PHYLIP-phylogeny interference package (version 3.2). Cladistics 1989;5:164—6.
[10] Taliani G, Guerra E, Rosso R, Badolato MC, Luzi G, Sacco G, et al. Hepatitis C virus infection in hypogammaglobulinemic patients receiving long-term replacement therapy with intravenous immunoglobulin. Transfusion 1995;35:103—7.
[11] Mahale P, Kontoyiannis DP, Chemaly RF, Jiang Y, Hwang JP, Davila M, et al. Acute exacerbation and reactivation of chronic hepatitis C virus infection in cancer patients. J Hepatol 2012;57:1177—85.
[12] Erratum EASL. Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol 2014;60:392—420.
[13] Kisiel E, Radkowski M, Pawelczyk A, Horban A, Stanczak J, Bukowska-Ośko I, et al. Seronegative hepatitis C virus infection in patients with lymphoproliferative disorders. J Viral Hepat 2014;21:424—9.