has not been well characterized. Immediately after inoculation, HCV particles have been assumed to bind to hepatocytes, such that virus is primarily confined to the liver with minimal, if any, release into the blood stream. Our study indicates that viral dynamics in the early pre-ramp-up phase may follow one of two major patterns: in some viremia is undetectable in plasma until a sustained rapid increase in serum RNA levels occurs, whereas in others intermittent low-level HCV viremia appears to precede this burst in RNA levels. These two patterns were also reported by Nübling and colleagues who found PCR-positive donations with RNA levels of less than 300 IU per mL preceding PCR-negative donations in the pre-ramp-up phase in 10 of 40 panels evaluated.[7] These low-titer viremic episodes were observed between 7 and 21 days before RNA levels exponentially increased, whereas we observed such events as early as 63 days before ramp-up. This difference may be the result of more sensitive, replicate testing of pre-ramp-up specimens in our study, indicating that the rate of detection and duration of detectable pre-ramp-up viremia will be impacted by study design and RNA assay sensitivity. It should be noted that neither study can estimate the duration of the pre-ramp-up phase because the date of exposure in infected plasma donors was unknown. The frequency with which individuals will develop one pattern versus another is also uncertain because these plasma donor sample panels were not randomly selected (see Materials and methods) and samples were not obtained on a daily basis. Hence, 74 percent (37/50) of the panels included in our study exhibited intermittent low-titer viremia compared to 25 percent (10/40) of panels evaluated by Nübling and coworkers.[7]

Reports of intermittent viremia in early HCV infection, that is, before seroconversion occurs, are not limited to plasma donor panels because this phenomenon has also been observed in immunocompromised patients,[17] injection drug users,[18,19] and after inoculation of chimpanzees.[1] The panels evaluated in our study were derived from otherwise healthy plasma donors whose infections were community-acquired (persons who received transfusions are deferred from donation for 1 year). Although injection drug use results in permanent donor deferral if reported at time of donation, we still believe that a proportion of the plasma donors included in our study acquired HCV though injection drug use because it is the most common HCV risk factor in the United States[20] and because some high-risk donors may not acknowledge this behavior at the time of donation. Supporting this assumption is the finding that 42[21] and 29 percent[22] of blood donors who had denied drug use at the time of their HCV antibody- or RNA-positive donation, respectively, acknowledged on recontact that they had injected drugs before their donation. It is therefore of interest that the low-grade intermittent viremia that was observed in some plasma donors in our study appears to be consistent with observations made by Beld and coworkers[19] who reported that low levels of serum HCV RNA could be intermittently detected for prolonged periods (>1 year) before HCV seroconversion in HIV-negative injection drug users.

The pre-ramp-up viremic episodes could result from intermittent detection of a persistent, low-titer viremia that occurs throughout the eclipse or pre-ramp-up phase. Conversely, it is possible that viremic periods alternate with nonviremic periods as a result of intermittent release of viral particles in the serum from replicating sites, that is, the liver and possibly peripheral blood cells.[23] Alternatively, these episodes could represent repeated exposures or reinfections that usually abort, until a particularly fit variant is finally transmitted and takes hold. Different genotypes have been identified at various time points in the same injection drug user suggesting that multiple exposures and reinfection can occur in this high-risk population.[19] Further studies comparing the genetic sequences of virus detected during the pre-ramp-up phase with virus detected during the ramp-up and plateau phases would be of interest to resolve these alternative hypotheses. Unfortunately, the levels of HCV RNA are extremely low (detectable only by high sensitivity, qualitative NAT assays) during the pre-ramp-up phase, such that generation of sufficient RNA for sequence analysis has proven exceedingly difficult (we have thus far not been successful in deriving sequence data from TMA-positive pre-ramp-up specimens).

The reasons for individual variations in the duration and viremic patterns of very early HCV infection are not known, but it is likely that the route of entry (percutaneous vs. mucosal), the nature of the inoculum (e.g., volume, viral concentration, and genetic diversity), the frequency of exposure, and the host immune response play key roles. HCV infection is characterized by genetic diversity. The evolution of the infection is related to the strength of the host's humoral and cellular immune response. Investigators[24,25] have conjectured that a sufficiently strong immune response can control and clear escape variants with resolution of the disease, whereas a moderate response may only partially control the infection resulting in escape of mutant variants and chronic infection, a hypothesis supported by several reports that have evaluated T-cell responses in acute and chronic HCV infection.[26,34]

Finally, the presence of low-titer viremia in the pre-ramp-up phase of some individuals raises the possibility that secondary transmission could occur from recently infected persons in the pre-ramp-up phase of very early HCV infection and supports retention of the current 12-month HCV lookback policy. These findings could impact estimates for the risk of transmission through blood transfusions or organ or tissue transplant. In the United States, this risk of transfusion-transmitted HCV has been estimated at 1 in 1.9 million donations for HCV since

minipool NAT implementation in 1999.[10,35,36] This calculation has assumed that the window period during which a donation can be infectious is the period between the onset of ramp-up and detection by minipool NAT and has therefore ignored the possibility that individuals may be viremic and potentially infectious for a prolonged period before the ramp-up phase. Rare instances of HCV transfusion transmission have been reported in recipients of minipool NAT–negative blood products.[37,38] In one case, the blood product was later found to be positive on individual NAT testing, implying that the viral load was between 50 and 600 IU per mL.[37] In the second case, the plasma of the transmitting donation was found to be positive in only one of several individual NAT assays indicating that the viral load was probably less than 10 geq per mL.[39] The low viral load in the latter case is consistent with what we observed in the pre-ramp-up phase and would suggest that donors with low-level viremia potentially could be infectious. Hence, the infectivity potential of these low-level viremic episodes needs to be evaluated, and chimpanzee studies are in progress with plasma donation specimens from the current study to assess whether these low-viral-load inocula can transmit infections. Until additional data are acquired, we recommend that the residual risk of HCV infection continue to be calculated as it has been, considering only the contributions of ramp-up infections.

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APPENDIX

Construction of the HCV-positive plasma donor panels

Plasma donations (600-800 mL) from source plasma donors were routinely collected at donor centers operated by Alpha Therapeutic Corporation (Los Angeles, CA) at approximately twice-weekly intervals. The collected plasma was frozen within 8 hours of collection and routinely stored frozen at not greater than –20°C for at least a 60-day quarantine period. Samples of plasma from each donation were submitted for infectious disease screening including serologic assays and HCV RNA testing in pools of 512 with a sensitive HCV RT-PCR assay (National Genetics Institute, Los Angeles, CA). HCV infection was confirmed by follow-up RNA testing and antibody seroconversion on undiluted samples from individual donors. HCV RNA–positive donors were notified, counseled, and permanently deferred. Frozen plasma donations from cases with confirmed viremia or seroconversion were retrieved from quarantine to construct panels comprised of sequentially drawn plasma samples. Each donation was handled separately in laminar flow hoods with single-use disposable equipment and other precautions to prevent possible cross-contamination. Units were rapidly thawed to room temperature, mixed, and aliquoted into 50-, 10-, and 2-mL aliquots, which were immediately refrozen at not greater than –20°C. Serial donation aliquots were coded and compiled into anonymized panels, not linked to individual donors. The records of each donation date and the results of routine and research laboratory tests for each plasma aliquot were entered in a computerized database that included the anonymized study codes.

Imputing the number of viral copies per mL present in units collected before RNA could be quantified

We imputed the number of viral copies per mL present in units collected before Time 0 (i.e., before RNA could be quantified by Amplicor Monitor PCR) from the number of positive TMA replicate results. The sensitivity curve associated with the d-HCV TMA assay has been estimated by Giachetti and associates[11] who reported 50 percent sensitivity levels (or viral loads at which individual NAT has a 50% chance of resulting in a positive test result) of 13.2 copies per mL.[11] We imputed that a sample that was positive on two of the four TMA replicates (50%) had a viral load of 13.2 copies per mL, that is, the 50th percen-

tile value of the d-HCV TMA sensitivity curve. Similarly, samples that were positive on one of four replicates were imputed to have for viral load the 25th percentile (8.9 copies/mL), whereas the viral load of samples positive on three of four replicates was imputed to be the 75th percentile (19.6 copies/mL) of the sensitivity curve. Finally, samples that were reactive on all four replicates were imputed to have a viral load of 119.2 copies per mL, which was the geometric mean of 23.7 copies per mL (the value from the sensitivity curve at which there is a 50% chance of having four of four positive TMA replicates) and 600 copies per mL (the inferred limit of detection for the Amplicor Monitor PCR; see package insert.).

Selecting the ramp-up bleeds

Assuming that the first RNA quantifiable bleed (Time 0 bleed) probably occurred in ramp-up and that viral load increased at a constant rate (i.e., in a linear fashion) on a log scale during ramp-up, we calculated the rate of increase in log viral load observed 1) between the bleed preceding the Time 0 bleed and the Time 0 bleed and 2) between the Time 0 bleed and the following bleed. Because the rate of increase in log viral load for each panel was the slope of the lines joining the two bleeds of interest (shown in Fig. 2), we obtained a pre-Time 0 and post-Time 0 slope for each panel. The combined distribution of all pre-Time 0 and post-Time 0 slopes was bimodal. This combined distribution supposedly represented the mixture of two distributions: 1) a distribution of larger slopes (fast rate of increase) corresponding to bleeds given in the ramp-up phase and 2) a distribution of smaller slopes (low rate of increase) corresponding to bleeds given in the pre-ramp-up or plateau phases. Discriminant analysis separated the two distributions from one another at a slope value of 0.3. Hence, we assumed that bleeds associated with a slope of less than 0.3 occurred in the pre ramp-up or plateau phases whereas bleeds associated with a slope of 0.3 or more occurred in the ramp-up phase.

Conservatively back-estimating when ramp-up started with a cutoff of 0.05 copies per mL

The ramp-up start day was determined by back-calculating, with each donor's specific rate of increase in log viral load during ramp-up, the day on which viral load would have been 0.05 copies per mL. We considered the following factors when selecting this extremely low viral load level to delineate the pre-ramp-up from the ramp-up phase. First, we estimated that a sample of 7.4 viral copies per mL would have a 50 percent probability of yielding no reactive TMA results (zero of four) based on the TMA test sensitivity reported by Giachetti and coworkers.[11] Thus at onset of ramp-up, the viral load was likely to be less than 7.4 copies per mL if the last pre-ramp-up phase bleed yielded all negative TMA results. Hence, to conservatively estimate the day when ramp-up started, we wanted to back-calculate to a viral load below 7.4 viral copies per mL. Second, although viral load is supposedly monotonically increasing during early ramp-up, the high rate observed in later ramp-up is probably not yet attained in early ramp-up. Hence, our back-estimation with the rate of increase in log viral load observed in later ramp-up could underestimate when ramp-up started if we had selected a viral load just below 7.4 copies per mL. We therefore decided to choose a viral load well below 7.4 copies per mL to likely ensure that we would not classify a ramp-up bleed as a pre-ramp-up bleed in our analysis. Finally, a serum RNA level of 0.05 viral copies per mL holds a special meaning in transfusion medicine because it corresponds to the numbers of copies per mL that would be present in 1 unit of RBCs that contains just one HCV viral copy (a RBC unit processed with the additive-solution procedures commonly used in the United States contains approx. 20 mL of plasma).

This back-calculation is not meant to infer that serum RNA level is at 0.05 viral copies per mL at the onset of the ramp-up phase. Rather, at onset, the level is likely to be less than 7.4 copies per mL if the last pre-ramp-up phase bleed yields all negative TMA results and is likely to be more than 7.4 copies per mL if the last pre-ramp-up phase bleed yields some reactive TMA results. Conservatively estimating the day when ramp-up started meant that a late pre-ramp-up bleed was likely to be characterized as an early ramp-up bleed. This approach, however, allowed us to be reasonably certain that we had not included a ramp-up bleed in our pre-ramp-up classification.

Selecting the plateau-phase bleeds

Because we did not have test information on a daily basis, we were unable to ascertain the exact date of antibody seroconversion nor the exact date when ramp-up ended and plateau phase began (such data are known as interval-censored data).[10] We therefore assumed that the plateau phase began on some day between the last "definitive" ramp-up phase bleed and the first definitive plateau phase bleed and assumed that seroconversion occurred on some day between the first antibody-positive bleed and the preceding bleed. For the 55 panels, we defined the last ramp-up unit that was selected in our analysis of the ramp-up phase as the definitive last ramp-up phase bleed. The ensuing first bleed with lower viral load than the previous bleed was the definitive first plateau phase bleed. For the supplemental 22 panels, the last bleed not detected by RT-PCR (conducted on pools of 512) was defined as the definitive last ramp-up–phase bleed whereas the definitive first plateau phase bleed was the first bleed with viral load greater than 5.0×10^4 copies per mL. ■

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研究報告の概要

○静脈瘤硬化療法中のC型肝炎ウイルス患者間感染の疫学的及び系統発生学的証拠
HCVジェノタイプ2の感染の証拠が認められる43名の患者が、同一医師による静脈瘤硬化療法を受けていた。この観察結果に基づき、HCVジェノタイプ2感染患者におけるHCV危険因子に関する調査票による詳細な疫学的調査を行った。硬化療法歴のあるHCV-RNA陽性患者17名から得たHCVゲノムの超可変領域1（HVR1）の17個の配列を、硬化療法歴のないジェノタイプ2の患者2名から得た17個の配列、またGenBankから抽出した25個の配列と比較した。207名のHCVジェノタイプ2感染患者を調査対象とした。HCV感染の主な危険因子は輸血（n=76）、薬物使用（n=6）、静脈瘤硬化療法（n=62。そのうち43名[20.8%]が同一医師による）、その他または不明（n=76）であった。これらの硬化療法セッションは、1980年代の数年間にもわたって行われた。これらの患者43名中5名で、硬化療法の数週間後に黄疸が認められた。同一医師による硬化療法を受けた17名の患者のHVR1の配列解析から、全員がHCVジェノタイプ2cに感染していたことが明らかになった。系統樹からは、硬化療法歴のある患者のクラスター化が示された。感染原因は、複数の患者に対して単一のバイアルを使用したことであったと考えられる。本研究により、静脈瘤硬化療法はHCV感染の危険因子であることの強力な証拠が示される。

報告企業の意見	今後の対応
静脈瘤硬化療法中に複数の患者に対して単一のバイアルを使用したことにより、C型肝炎ウイルス（HCV）の院内感染が発生したと考えられるとの報告である。輸血後HCV感染症の調査には、院内感染など輸血以外の伝播ルートについて考慮する必要がある。	HCV感染の新たな伝播ルート等について、今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Journal of Medical Virology 76:279–284 (2005)

Epidemiological and Phylogenetic Evidence for Patient-to-Patient Hepatitis C Virus Transmission During Sclerotherapy of Varicose Veins

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The aim of this study was to provide evidence for patient-to-patient nosocomial hepatitis C virus (HCV) transmission during sclerotherapy of varicose veins. Forty-three patients who had evidence of current infection by genotype 2 HCV have had sclerotherapy by the same physician. Based on this observation, a detailed epidemiological questionnaire on risk factors for HCV in genotype 2 infected patients was conducted. Seventeen sequences in the hypervariable region 1 (HVR1) of the HCV genome obtained from 17 HCV RNA positive patients with a past history of sclerotherapy, were compared with 17 sequences derived from genotype 2 patients with no past history of sclerotherapy, and with 25 sequences sampled from GenBank. Two hundred seven genotype 2 HCV infected patients were included. The main risk factors for HCV infection were transfusion (n = 76), drug use (n = 6), and sclerotherapy of varicose veins (n = 62 including 43 (20.8%) by the same physician), other or unknown (n = 76). These sclerotherapy sessions were carried out in the 1980s for many years. Five of these 43 patients had jaundice within a few weeks after a sclerotherapy session. Sequence analysis of HVR1 from 17 patients who had sclerotherapy by the same physician revealed that they were all infected with HCV genotype 2c. The phylogenetic tree indicated clustering of the patients with a past history of sclerotherapy. The method by which infection was likely to have been transmitted was probably the use of a single vial for multiple patients. This study provides strong evidence that sclerotherapy of varicose veins is a risk factor for HCV infection. ***J. Med. Virol. 76:279–284, 2005.*** © 2005 Wiley-Liss, Inc.

KEY WORDS: hepatitis C virus infection; nosocomial infection; HCV outbreak; patient to patient infection

INTRODUCTION

Nosocomial infection with hepatitis C virus (HCV) is well documented in about 30% of HCV-infected patients and may occur in a variety of circumstances [Sanchez-Tapias, 1999]. Due to the characteristically silent course of HCV infection, many nosocomial infections may be unrecognized and when reported, they usually reflect a breakdown in infection control measures. Retrospective or prospective surveys of patients with chronic hepatitis C have shown that therapeutic injections with nondisposable syringes and needles were risk factors for HCV infection [Chen et al., 1995]. Several instances of transmission in healthcare settings have been documented. It has been suggested that the vehicles for transmission include contaminated anesthetic breathing circuits, or inadequate cleaning of endoscopic equipment [Bronowicki et al., 1997], faulty administration of intravenous anesthetic drugs [Tallis et al., 2003], multidose vials [Widell et al., 1999; Lagging et al., 2002]. Transmission of HCV from a patient to a surgeon or an anesthesiology assistant [Ross et al., 2000], and from a surgeon to his patients have been also documented [Esteban et al., 1996]. However, to our knowledge, transmission of HCV during sclerotherapy of varicose veins has not been reported.

Epidemiological data and phylogenetic analysis indicate the transmission of HCV during sclerotherapy of varicose veins and the clinical, virological, and histological outcome in these patients are described.

The first two authors contributed equally to the study.

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PATIENTS AND METHODS

Between 1996 and 1998, five cases of HCV-infected patients with a past history of sclerotherapy of varicose veins were noted. All the patients had been treated by the same physician and were infected by the same genotype 2 virus suggesting a common source of infection. Due to this observation, a study was conducted using a detailed epidemiological questionnaire on risk factors for HCV in genotype 2 infected patients in charge in our Hepatology unit from January 1998 to December 2002.

All patients were interviewed using the epidemiological questionnaire on risk factors for HCV infection listed in Table I. In case of sclerotherapy of varicose veins, the number of sessions, the beginning and ended years of sessions, and the name of the physician who carried out these sessions, were recorded.

Demographic information was collected including gender, age, alcohol, and tobacco consumption. Other obtained information included biochemical marker of liver injury (serum alanine aminotransferase ALT) and hepatitis B virus (HBV) and human immunodeficiency virus (HIV) serostatus. When indicated, a liver biopsy was undertaken. The histological grade of disease activity and the histological stage of fibrosis were assessed using the METAVIR scoring system [Bedossa and Poynard, 1996].

HCV infection was defined as detectable HCV antibodies by the ELISA 3.0 HCV assay (Ortho Clinical Diagnostics, Raritan, NJ). 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 (Cobas Amplicor HCV 2.0, Roche Diagnostic, Branchburg, NJ) [Young et al., 1993].

TABLE I. Risk Factors for HCV Infection Recorded in the Epidemiological Questionnaire

Blood transfusion (including platelet or plasma transfusion, previous administration of coagulation factor concentrates)
Intravenous drug use
Cocaine use
Previous endoscopic examination
Previous coelioscopy examination
History of surgery
History of acupuncture
History of mesotherapy[a]
Voluntary termination of pregnancy
Immunoglobulin injection
Transplantation
Hemodialysis
Length of hospitalization for more than 15 days
History of incarceration
Institutional living
Tatoo or body piercing
Health profession
Infection of the spouse
Sclerotherapy of varicose veins

[a]Mesotherapy is a technique where small amounts of medication are injected into the connective tissue to stimulate the mesoderm for various biological purposes.

Quantitation of HCV RNA was performed with a commercial branched-chain-DNA signal-amplification assay (Versant HCV 3.0, Bayer Diagnostics, Eragny, France).

HCV genotyping was carried out by sequence analysis of the 5′ noncoding region of the genome (Trugene 5′ NC HCV genotyping kit; Visible Genetics, Toronto, Canada).

In order to determine if the virus had been transmitted between the HCV genotype 2 patients, sequence analysis was undertaken of part of the HVR1 regions of the genomes in samples of patients with past history of sclerotherapy of varicose veins. The HVR1 fragment (nucleotide positions 1,329–1,586) was chosen for sequence analysis because this domain exhibits a sufficiently high degree of variability to allow analyses to distinguish between HCV isolates of the same subtype. The PCR protocol used for amplification of the HVR1 region has been detailed previously [Larghi et al., 2002]. Two-strand direct sequencing was carried out on the nested PCR products of 258 bp (nt 1329–1586). These products were purified with QIAquick PCR purification kit (Qiagen, Chatsworth, CA) and sequenced subsequently in the sense and antisense directions by the dideoxy chain termination method using CEQ DTCS Quick Start Kit on an automated sequencer Beckman CEQ 2000 DNA Analysis System [Mondelli and Silini, 1999]. For phylogenetic analysis, 17 sequences, each 258 nt long, randomly selected from 17 of the 43 HCV RNA-positive patients who had sclerotherapy by the same physician were compared with 17 sequences (three of genotype 2a, five of genotype 2b, and nine of genotype 2c) derived from patients from the same geographical area, and without a past history of sclerotherapy of varicose veins taken as unrelated controls, with 20 sequences (19 of genotype 2c and 1 of genotype 2a) derived from a previous study by Larghi et al. [2002] and with 5 prototype sequences sampled from GenBank (D00944 and D10075 for genotype 2a, D10077 and D10988 for genotype 2b, D50409 for genotype 2c). Viral sequences were first aligned using CLUSTAL W sofware, version 1.74 [Thompson et al., 1994]. The sequences were gap-stripped and the pairwise matrix was generated. Phylogenetic trees were inferred using neighbor-joining method from matrix distances calculated after gapstripping of alignments, with Kimura two-parameter algorithm. The final unrooted consensus tree was drawn with TREEVIEW version 1.4 program [Power et al., 1995]. The whole set of 59 sequences was resampled by bootstrapping 100 times with CLUSTAL W. The numbers at the nodes indicate the frequency with which the node occurred in 100 bootstrap replicates.

Differences in frequencies were compared using the Chi-square or Fisher's exact test, as appropriate. Differences between two means were compared using the Student's *t*-test. Logistic regression was used to identify variables that could be associated with a past history of sclerotherapy of varicose veins. A two-tailed critical *P*-value of <0.05 was used throughout.