CONCISE COMMUNICATION

Nosocomial Outbreak of Multiple Bloodborne Viral Infections

Sabine Yerly,1 Rafael Quadri,2 Francesco Negro,2,3 Klara Posfay Barbe,1 Jean-Jacques Cheseaux,3 Philippe Burgisser,6 Claire-Anne Siegrist,4 and Luc Perrin1

In resource-limited countries, nosocomial transmission of bloodborne pathogens is a major public health concern. After a major outbreak of human immunodeficiency virus (HIV) infection in ~400 children in 1998 in Libya, we tested HIV, hepatitis C virus (HCV), and hepatitis B virus (HBV) markers in 148 children and collected epidemiological data in a subgroup of 37 children and 46 parents. HIV infection was detected in all children but one, with HCV or HBV coinfection in 47% and 33%, respectively. Vertical transmission was ruled out by analysis of parents’ serology. The children visited the same hospital 1–6 times; at each visit, invasive procedures with potential blood transmission of virus were performed. HIV and HCV genotypic analyses identified a HIV monophyletic group, whereas 4 clusters of HCV sequences were identified. To our knowledge, this is the largest documented outbreak of nosocomial HIV transmission.

Transmission of bloodborne viral pathogens through invasive medical procedures has been long recognized [1]. In industrialized countries, invasive medical procedures were major routes of pathogenic spread until some decades ago [2], and occasional nosocomial outbreaks are still reported [3, 4]. In countries with limited health care resources, the higher prevalence of parenterally transmissible pathogens, the overuse of injections, the frequent sharing of syringes, and the poor enforcement of safety guidelines are associated with frequent outbreaks of bloodborne pathogens [1, 5]. In these countries, children are at higher risk because of their frequent use of medical facilities and because of the paucity of symptoms in most patients during acute human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV) infection.

The Libyan representative of the World Health Organization (WHO) alerted us of the occurrence of ~400 recent cases of HIV infection among children attending the Pediatric Department of Al-Fateh Hospital (Benghazi, Libya) in 1998 and asked us to perform in-depth investigations on 111 coded plasma samples and on a group of infected children referred to us. Our investigation was limited to virological analysis and to the collection of basic epidemiological data, because we did not have the opportunity to complete our investigation by a field study.

Methods

Study participants. All children were treated at the Al-Fateh Hospital, where the first case of HIV infection was detected in May 1998. In September 1998, 111 coded plasma samples from HIV-infected children were provided to us. Epidemiological data were collected in a group of 37 children and 46 of their parents referred to the Geneva and Lausanne University Hospital by the Libyan government in April 1999.

Serology. Anti-HIV, anti-HCV, anti–hepatitis B core antigen (HBcAg), anti–hepatitis B surface antigen (anti-HBsAg), and hepatitis B surface antigen (HBsAg; Assym) were measured in plasma of parents’ serology. The children visited the same hospital 1–6 times; at each visit, invasive procedures with potential blood transmission of virus were performed. HIV and HCV genotypic analyses identified a HIV monophyletic group, whereas 4 clusters of HCV sequences were identified. To our knowledge, this is the largest documented outbreak of nosocomial HIV transmission.

Received 26 January 2001; revised 6 April 2001; electronically published 10 July 2001.

Informed parental consent was obtained, and ethical committees approved the investigation.

Financial support: Swiss National AIDS Research Program (grant 3200-56.059.98); Swiss National Science Foundation (grant 32-52193.97); Libyan government.

Reprints or correspondence: Dr. Luc Perrin, Laboratory of Virology, Geneva University Hospital, 1211 Geneva 14, Switzerland (luc.perrin@hcuge.ch).

The Journal of Infectious Diseases 2001; 184:369–72
© 2001 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/2001/18403-0018$02.00
a commercial assay (INNO-line probe assay HCV II; Innogenetics NV).

**HCV E2 amplification and sequencing.** Total RNA was extracted from plasma and was reverse transcribed. The hypervariable region 1 of the envelope glycoprotein E2 (nt 952–1365) was amplified with primers specific for genotypes 1 and 4 (5'-ATGGGCNTGGGAYATGATGAAATG-3' and 5'-CGGYTCGGRRCASCTGARSWTGTTAYTTGT-3') [9], which was followed by a nested polymerase chain reaction (PCR) with primers 5'-GGGBCATGTGGHRTCT-3' and 5'-ATRTGCARCTSCCRRTGSTGGA-3' (nt 1048–1265). Each round of PCR consisted of a 5-min hot start at 95°C, followed by 35 cycles with a denaturation step at 94°C for 30 s, an annealing step at 55°C for 45 s, an elongation step at 72°C for 45 s, and a last extension step at 72°C for 7 min. Direct sequencing was performed with the primers used for the nested PCR.

**Phylogenetic analysis.** Sequences encompassing bp 861–1246, bp 2252–2549, bp 2634–3223, and bp 6958–7281 of the HIV 1 gag region were estimated with the DNADIST program. Evolutionary distances were estimated by using the PHYLIP software package [11]. Phylogenetic analyses were carried out by using the PHYLIP software package [11]. Evolutionary distances were estimated with the DNADIST program. Trees with all branch lengths drawn to scale were constructed by the NEIGHBOR program.

**Results**

**Epidemiological data.** According to the Libyan WHO representative, 393 children were infected with HIV in 1998 at the Pediatric Department of Al-Fateh Hospital. Appropriate measures were implemented in 1999 to stop this outbreak, including the use of disposable injection material. A detailed history, which was collected from the 37 children and the 46 parents referred to us, revealed that all these children had been seeking medical care as outpatients (n = 14) or had been hospitalized (n = 27) at the Al-Fateh Hospital. The mean number of visits from January 1998 through April 1999 was 2.14 visits (range, 1–4 visits) for outpatients and 1.56 visits (range, 1–6 visits) for hospitalized patients. All children had invasive procedures for blood collection, and most of them had a venous line. Vaccination history was documented for all children; 83% had been vaccinated against HBV during the first 6 months of life.

**Serological analysis in children and parents.** The data on the 111 coded plasma collected in September 1998 are reported in table 1. HIV antibodies were detected in 99%, and HIV-1 infection was confirmed by Western blot (at least 1 positive band for both env and gag components) in 96%. Two samples without antibodies were positive for HIV-1 RNA. Twenty children had a Western blot pattern suggestive of primary HIV infection (<4 reactive bands).

Anti-HCV was detected in 46% of the plasma samples. HCV RNA was detected in 3 of 5 anti-HCV−negative samples from patients with recent HIV infection (<4 positive bands on Western blot), which suggests HIV-HCV coinfection in some of the children. Anti-HBsAg, anti-HBcAg, or both were detected in 56%, 31%, and 12.5%, respectively, of the plasma samples. Eighteen percent of the samples had anti-HBcAg without anti-HBsAg. The small amount of plasma available precluded a systematic dosage of HBsAg. Overall, 22% of the children were coinfected or had been infected with HIV, HCV, or HBV.

Table 1 also reports the serological data from the groups of 37 children and 46 parents referred to us. The mean age of these children was 4.1 years (range, 0.7–15.8 years). HIV-1 infection was confirmed by Western blot. Anti-HCV antibodies were detected in 43%. Anti-HBsAg and anti-HBcAg were detected in 59% and 33% of the plasma samples, respectively. Eleven percent of the study group had anti-HBcAg without anti-HBsAg. Of the 31 children vaccinated against HBV during the first 6 months of life, 6 had both anti-HBsAg and anti-HBcAg antibodies. HBV serology was available from the mothers of 3 of them; none were positive for HBsAg or had isolated anti-HBcAg. The prevalence of HCV and HBV serological markers in this group of children was similar to that of the coded plasma group. The parent’s serology (25 mothers and 21 fathers) revealed that only 1 mother was positive for anti-HIV and that 2 parents were positive for anti-HCV (the mother of a anti-HCV−negative child and 1 father). Anti-HBcAg was detected in 26% of the parents, and all but one (HBsAg positive) had anti-HBcAg.

**Genetic characterization of HIV.** HIV sequences were determined in 36 children. Neighbor-joining trees including these sequences and reference sequences of HIV-1 group M (subtypes A, B, C, D, G, and CRF02_ A/G) revealed that sequences from children form a monophyletic group with bootstrap values beyond 70% confidence.
Figure 1. A, Phylogenetic trees constructed from human immunodeficiency virus type 1 (HIV-1) p17 \textit{gag} sequences from 36 children. Phylogenetic analysis was carried out by using PHYLIP software package [11]. Bootstrap scores $\geq 700$ (70% of 1000 replicates) are shown. Reference sequences from 5 HIV-1 (U455-A, HXBR-B, 92BR25-C, ELI-D, and 92NG083-G) and 1 circulating recombinant form (CRF02, A/G) group M subtypes are reported. B, Phylogenetic trees constructed from hepatitis C virus (HCV) E2 envelope glycoprotein sequences from 15 Libyan children. Phylogenetic analysis of the HCV E2 encoding region, which was carried out by using the PHYLIP package, was based on comparison of representative nucleotide sequences from HCV genotypes 1a, 1b, 2, and 4a and the 15 children's isolates [11]. Bootstrap scores $\geq 700$ (70% of 1000 replicates) are shown. Four different clusters are observed, which are composed of 8 isolates in cluster 1 (6415, 4702, 4449, 7300, C03, C04, 7273, and A33), 1 in cluster 2 (A32), 3 in cluster 3 (B01, 6529, and 3082), and 3 in cluster 4 (6534, C02, and 3205). Clusters 1, 2, and 2 belonged to genotype 4, and cluster 4 belonged to genotype 1a.

Data analysis

Recent modeling suggests that $\approx 8$–16 million HBV, 2.3–4.7 million HCV, and 80,000–160,000 HIV infections occur yearly through unsafe injections worldwide, mostly in resource-limited countries [1]. In this latter setting, our study reports the largest outbreak of nosocomial transmission of HIV infection associated with a high incidence of HCV and HBV transmission.

Our investigation was limited by the lack of access to a field study, which could have provided more detailed epidemiological informations. However, the nosocomial origin of HIV infection is supported by the monotypic characteristic of the HIV sequences. The interisolate variation for the p17 \textit{gag} region among Libyan children (0%–2.4%) was lower than the 4.5%–17% reported elsewhere for unrelated isolates [12]. Higher interisolate variation was observed for the C2V3 region (0%–9%), which probably reflects a higher substitution rate due to immune pressure, but this variability corresponds to that reported for related samples within sexual or parenteral transmission [13].

However, these genetic data should be interpreted with caution, because, during the rapid spreading of HIV, low variation among HIV strains of unrelated infected people has been reported [14]. Other arguments support the nosocomial origin of this outbreak: all children attended the Pediatric Department of the same hospital and underwent invasive procedures; the serological data of a subgroup of parents exclude vertical transmission; there is a high incidence of concomitant HIV, HCV, and even HBV infection, despite an active HBV vaccination.
program; and evidence suggests that some children were coinfected with HIV and HCV during the same invasive procedure.

The low HIV interisolate variation contrasts with the detection of 4 HCV clusters. This observation is consistent with the very low prevalence of HIV infection, compared with the high prevalence of HCV infection in Libya and neighboring Egypt; this could, in turn, result in multiple entries of HCV but not of HIV [5, 15]. The most likely scenario is that a first child was infected with HIV through contaminated injection material or through an unidentified vertical transmission. Injection material contaminated by his blood then generated the spread of HIV infection. Ongoing acute primary HIV infection, with several children carrying very high viremia, then would have contributed to the explosive spread of HIV infection. Consistent with this hypothesis, 20 children had a Western blot profile consistent with primary HIV infection in 1998, and 4 of the children referred to us had a viremia >10^6 HIV-1 RNA copies/mL (data not shown). Finally, the selection of the children’s population, which was based on a positive HIV serology, did not exclude a much larger parallel outbreak of HCV infection, which should be investigated.

The spreading of HBV infection probably was limited by the high percentage of children vaccinated in the first 6 months of life. However, evidence of HBV infection, which was based on detection of anti-HBcAg antibodies, was present in 33% of children. More importantly, the plasma samples of 6 children, who were vaccinated before the age of 6 months, contained both anti-HBsAg and anti-HBcAg antibodies. Because vertical transmission was ruled out for 3 of them, their serological pattern is thus consistent with an HBV contamination during or close to the admission of the HBV vaccine.

Detection of this large outbreak was possible though the commitment of the Libyan authorities to assess its extension and to stop it. It underlines the necessity to invest in education programs for health care workers and to enforce safe medical practices.

Acknowledgments

We thank Amer Rahil, Libyan representative of the World Health Organization, for providing us with information about the outbreak; Covance Laboratory in Geneva for providing us with plasma samples; Bernard Hirschel (AIDS Unit, Geneva University Hospital, Geneva, Switzerland) and Peter Grob (Clinical Immunology, Zurich University Hospital, Zurich, Switzerland) for helpful discussion; David Nadal (Pediatric Department, Zurich University Hospital) for coordination of the admission of Lybian children in Switzerland; and Wanda Caveng, Chantal Gaille, Patrick Schreiber, and Isabelle Vernez for technical work.

References