Case Report

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Detection of HIV-1 Infection in Dried Blood Spots from a 12-Year-Old ABO Bedside Test Card

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Abstract

Background and Objectives: We tested dried blood from an ABO bedside test card which had been stored at room temperature for 12 years, to prove that a patient with HIV-1 infection had been infected by blood transfusion. **Materials and Methods:** Immunoblots for HIV-1 antibodies and threefold PCRs with half-nested primers for the HIV-1 integrase gene were done with eluates from the dried blood spots. **Results:** HIV-1 antibodies and HIV-1 DNA could be detected in the sample from one unit of blood, but not from the two other units or from the recipient before transfusion. **Conclusion:** Further studies should be done on the validity of stored dried blood as an alternative to the storage of frozen donor serum for several years for 'look-back' studies.

Introduction

Before 1985, when anti-HIV screening of blood donations was introduced [1], in Germany several hundred people (not counting haemophiliacs) had been infected by blood products that had not been tested [2]. In 1994, guidelines were issued for the 'look-back' of transfusion-associated HIV, HBV, and HCV infections [3]. The aim was (1) to find all recipients of potentially infected blood donated by a person found to have seroconverted at a subsequent blood donation, and (2) to find the donor, who has infected someone by blood transfusion, in order to prevent further infections caused by blood donations of this donor and to find all other recipients of blood from this donor, who might also have been infected.

We present here the case of a patient whose blood transfusion was the only apparent risk for HIV infection. We also report our method of identifying the route of infection 12 years earlier.

Case Report

In 1996, a 59-year-old woman became ill with pneumonia for the third time in several months. A screening test for anti-HIV-1/2 was positive. In the HIV-1 immunoblot, antibodies against the following antigens were detected: p24 (weak), p32 (weak), gp41, p51, p55, p66, and gp120. The HIV-2 immunoblot showed weak reactions with the antigens p25 and p55. These were interpreted as cross-reactions by the HIV-1 antibodies.

After recovery from respiratory infection, the patient was transferred to our department for further treatment. On admission, she had 2,700 leucocytes/μl with 38% lymphocytes. The absolute count of CD4+ lymphocytes was low (31/μl). No other opportunistic infections were observed.

The only risk factor for HIV infection was transfusion of three units of blood during a surgical intervention in 1984, when anti-HIV tests for donor screening were not yet available. Two of the donors involved were tested for HIV antibodies several years later at the time

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Table 1. Primers used for PCR to detect HIV-1 proviral DNA in eluates from dried blood spots from a 12-year-old ABO bedside test card

HIV gene	1st-round primers	2nd-round primers	3rd-round primers
Double PCRs			
gag	gaga:gagb	sk38:sk39	
pol-RT	pol3:pol4	pol3n:pol4n	
env	enva:envb	sk68:sk69	
pol-integrase	5pol201:3pol301	5pol211:3pol291	
Threefold PCR			
pol-integrase	5pol201:3pol301	5pol211:3pol301	5pol211:3pol291

Sequences of the primers (5′→3′) for the HIV-1 integrase gene:
5pol201: GTGAA TCAGA AATAG TCAGT C,
3pol301: ATCAT CACCT GCCAT CTG,
5pol211: CATGG GTACC AGCAC CACAA AG,
3pol291: AGTGG ATCTC TGCTG TCCCT G.

of further blood donations and were found to be anti-HIV-negative (donor 1 in 1987, donor 3 in 1988). Donor 2 was tested 1 year later (1985) and found to be anti-HIV-positive. In 1985, look-backs for recipients of such a donor's blood were unusual.

Materials and Methods

Blood spots from the recipient and the 3 donors had been preserved on a bedside test card with anti-A, anti-B and anti-D reagents in 1984. These were examined. The spots of dried blood on this card had been covered by an adhesive film and the card had been stored together with the patient's records at room temperature for 12 years.

The adhesive film was removed and the dried blood (2 of the 3 spots from each blood sample with a diameter of about 1.5 cm each) was scraped off and transferred together with a small part of the paper into a polypropylene tube.

To extract antibodies and remove haemoglobin, each dried blood sample was incubated with 1 ml lysis buffer (0.16 *M* NH_4Cl; 0.17 *M* Tris; pH 7.2) overnight at room temperature and for 2 h at 45°C. The supernatant was used for detection of HIV antibodies. A second elution procedure was done to remove most of the remaining haemoglobin.

Lysis buffer was removed by centrifugation and aspiration, subsequently 200 μl buffer A (100 m*M* KCl; 10 m*M* Tris; 2.5 m*M* $MgCl_2$; pH 8.3) and 100 μg proteinase K in 200 μg buffer B (10 m*M* Tris; 2.5 m*M* $MgCl_2$; 1% Tween-20; 1% NP-40) were added to each sample to liberate human and proviral DNA. After incubation at 65°C for 4 h (with continous shaking) and at 95°C for 5 min, the tubes were centrifuged for 7 min at 5,000 *g*. Each supernatant was diluted with distilled water to 1 ml and DNA was purified by QIAamp™ columns (QIAamp™ Kit, Diagen GmbH, Hilden, Germany) resulting in 50 μl DNA solution, from which 7 μl were used for each PCR test. HIV-1 antibodies were detected by an immunoblot technique using antigens from the HIV-1 isolate MVP-899 [4].

To demonstrate successful extraction of human DNA from the dried blood spots, a fragment of the chemokine receptor-5 (CKR-5) gene was amplified by PCR, as described by Liu et al. [5].

Several double PCRs with nested primers were used to detect proviral DNA for HIV-1 as described previously (table 1) [6]. As no amplification product was obtained, three half-nested PCRs were performed using primers of the HIV-1 integrase gene (table 1). For nucleic acid amplification by PCR, we chose the following conditions: 30 cycles for each round of replication, denaturation 30 s at 92°C, annealing 1 min at 55°C, and extension 1 min at 72°C. For the combination of 3 half-nested PCRs, an annealing temperature of 50°C was selected to obtain maximal sensitivity. In all PCR procedures, DNA extracted from cells infected with the HIV-1 isolate MVP-899 served as control.

Amplification products were subjected to electrophoresis in agarose gel (2.5%) and identified by ethidium bromide staining of DNA. Specificity was confirmed by agreement with the calculated molecular weight of the amplification product and by comparison with the amplification product of the control DNA.

Results

Antibodies to HIV-1 were found only in the lysis buffer extract of the dried blood spots of donor 2, not of the recipient or donors 1 and 3. The antibodies of donor 2 were directed against the following HIV-1 antigens: p32, gp41, p51, p55, p66 and gp120.

Succesful extraction of human DNA from all four dried blood samples was demonstrated by PCR amplification of a fragment of the CKR-5 gene with a molecular weight of 1,060 bp.

Double PCRs with nested primers for the HIV-1 proviral gag, env, pol-reverse transcriptase and integrase gene did not reveal any amplification product from the dried blood samples of the recipient and the three donors. Threefold PCRs with half-nested primers for the proviral integrase gene showed no specific amplification products from the

dried blood samples of the recipient and of blood donors 1 and 3. Only in the dried blood spot of donor 2 was the specific amplification product with the predicted molecular weight of 650 bp found. The detection of proviral HIV-1 DNA only in the blood sample of donor 2, not in those of the recipient (taken immediately before blood transfusion) and of the two other blood donors, confirms that blood from donor 2 was the source of the HIV-1 infection of our patient in 1984.

Discussion

Several studies have been reported on the use of spots of dried blood on filter paper for HIV testing (for example, of newborns or persons living in remote areas) by means of PCR [7–10]. Other reports describe the successful use of such blood spots in newborns for the detection of maternal HIV [11, 12] or HTLV-I [13] antibodies for epidemiological purposes. Also HBs antigen and other markers of HBV infection have already been detected in dried blood spots [14, 15].

Furthermore, dried sera on blotting paper have been transported over long distances for testing anti-HIV, anti-HTLV-I and anti-HCV [16, 17]. If experiments with more samples prove the validity of their results, Das et al. [17] propose to send dried sera of blood donors with positive results in screening tests from developing countries to reference laboratories in industrialized countries to perform confirmation tests.

As our study demonstrates, the examination of dried blood can also help to overcome lapses of time, since we identified the route of infection of a patient by means of HIV testing of spots of dried blood on a 12-year-old ABO bedside test card. This is not only of forensic interest, but may also have practical importance for blood donation services as Germany has regulations requiring storage of serum or plasma samples for at least 1 year after the expiration date of blood products [18].

For these reasons, we propose to carry out further studies on the validity of storing dried whole blood and dried serum or plasma for several years after blood donation as an alternative to the more expensive storage of frozen donor serum.

References

1 Richtlinie zum Nachweis von LAV/HTLVIII-Antikörpern bei Blutspenden. Bundesgesundheitsblatt 1985;28:122.
2 Hamouda O, Kiehl W, Voß L, Siedler A, Altmann D, Kollan C, Baars C, Kotzur KH, Meyer B, Schartländer B, Koch MA: AIDS/HIV 1994 – Bericht zur epidemiologischen Situation in der Bundesrepublik Deutschland zum 31.12.1994. Robert Koch Institut Berlin, 1995, pp 14–17.
3 Empfehlungen der Ad-hoc-Kommission des Arbeiterkreises Blut zum Rückverfolgungsverfahren (look back) für Einzelspender und Kleinpool-Blutpräparate. Bundesgesundheitsblatt 1994;37:513–514.
4 Gürtler LG, Eberle J, Lorbeer B, Deinhardt F: Sensitivity and specificity of commercial ELISA kits for screening anti-LAV/HTLV III. J Virol Methods 1987;15:11–23.
5 Liu R, Praxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, Landau NR: Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 1996;86:367–377.
6 Mueller AJ, Klauss V, Gürtler L, Geier S: Infrequent detection of HIV-1 components in tears compared to blood of HIV-1 infected persons. Infection 1992;20:249–252.
7 Cassol S, Salas T, Arella M, Neumann P, Schechter MT, O'Shaughnessy M: Use of dried blood spot specimens in the detection of human immunodeficiency virus type 1 by the polymerase chain reaction. J Clin Microbiol 1991; 29:667–671.
8 Cassol S, Salas T, Gill MJ, Montpetit M, Rudnik J, Sy CT, O'Shaughnessy MV: Stability of dried blood spot specimens for detection of human immunodeficiency virus DNA by polymerase chain reaction. J Clin Microbiol 1992;30:3039–3042.
9 Nyambi PN, Fransen K, de Beenhouwer H, Chomba EN, Temmermann M, Ndinya-Achola O, Piot P, van der Groen G: Detection of human immunodeficiency virus type 1 (HIV-1) in heel prick blood on filter paper from children born to HIV-1-seropositive mothers. J Clin Microbiol 1994;2858–2860.
10 Biggar RJ, Miley W, Miotti P, Taha TE, Butcher A, Spadoro J, Waters D: Blood collection on filter paper: A practical approach to sample collection for studies of perinatal HIV transmission. J Acquir Immune Defic Syndr 1997; 14:368–373.
11 Farzadegan H, Quinn T, Polk F: Detecting antibodies to human immunodeficiency virus in dried blood on filter papers. J Infect Dis 1987;155:1073–1074.
12 Peckham CS, Tedder RS, Briggs M, Ades AE, Hjelm M, Wilcox AH, Mejia PN, Connor CO: Prevalence of maternal HIV infection based on unliked anonymous testing of newborn babies. Lancet 1990;335:516–519.
13 Parker SP, Taylor MB, Ades AE, Hjelm M, Wilcox AH, Mejia PN, Connor CO: Use of dried blood spots for the detection and confirmation of HTLV-I specific antibodies for epidemiological purposes. J Clin Pathol 1995; 38:904–907.
14 Farzadegan H, Noori KH, Ala F: Detection of hepatitis B surface antigen in blood and blood products dried on filter paper. Lancet 1978;i: 362–363.
15 Villa E, Cartolari R, Bellentani S, Rivasi P, Casolo G, Manenti F: Hepatitis B virus markers on dried blood spots. A new tool for epidemiological research. J Clin Pathol 1981;34:809–812.
16 Das PC, de Vries AH, Shah S, Desai S, Antani D, McShine RL, Smit Sibinga CT: HIV-1/HIV-2 antibody confirmations by dry blood and sera transported on paper. Immunol Infect Dis 1995;5:266–269.
17 Das PC, de Vries AH, McShine RL, Smit Sibinga CT: Dried sera for confirming blood-born virus infections (HCV, HTLV-I, HIV & HBsAg). Transfus Med 1996;6:319–323.
18 Wissenschaftlicher Beirat der Bundesärztekammer und Paul-Ehrlich-Institut: Richtlinien zur Blutgruppenbestimmung und Blutransfusion (Hämotherapie). Bundesgesundheitsblatt 1996;39:473.