HIV-1 detection by nested PCR and viral culture in fresh or cryopreserved postmortem skin: potential implications for skin handling and allografting

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Abstract

***Aims*—To date, the risk relating to the handling or allografting of human immunodeficiency virus type 1 (HIV-1) infected postmortem skin remains hypothetical. While blood screening for HIV antibodies is still the key safety procedure to detect HIV infected cadavers, false negative results are a concern. Conversely, false positive results may hamper the collection of skin allografts. Accordingly, viral culture was used to clarify skin infectivity and the nested polymerase chain reaction (PCR) was used to assess the reliability of skin PCR testing.**

***Methods*—Viral culture and nested PCR performed with gag and pol specific primers were investigated in cadaveric skin and blood from 12 HIV-1 infected patients. Samples were collected repeatedly between one and five days in seven patients. In most cases, analyses were performed on triplicate skin samples: fresh (n = 26), cryopreserved in 5% dimethylsulphoxide (n = 21), or cryopreserved in 30% glycerol (n = 26).**

***Results*—HIV was isolated in two of 26 cultures of fresh skin specimens (8%), seven of 47 cryopreserved skin specimens (15%), and eight of 26 blood specimens (31%). The nested PCR detected HIV-1 in all skin samples (n = 73), regardless of the postmortem interval or cryopreservation. In blood, a positive signal was found in eight of 12 patients but two of them had discordant results on successive samples.**

***Conclusions*—These data suggest that nested PCR on postmortem skin samples can detect HIV more reliably than on blood. They also demonstrate the potential viral infectivity of fresh or stored skin postmortem samples in HIV infected patients. They underscore the need for caution during the handling of skin tissue from HIV infected cadavers and confirm the potential risk related to accidental allografting of HIV contaminated skin.**

(*J Clin Pathol* 1997;50:481–484)

Keywords: HIV-1; polymerase chain reaction; viral culture; postmortem skin; allograft

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Accepted for publication 24 March 1997

Postmortem skin is the main source of tissue for reconstructive surgery in patients treated for burns,[1] but handling and allografting skin specimens is hampered by the risk of human immunodeficiency virus type 1 (HIV-1) infection.[2] Blood screening for HIV antibodies, which has been implemented routinely since 1985,[3] remains the key procedure to detect HIV infected cadavers. The aim of donor selection is to offer the recipient maximum security by minimising the risk of false negative results, and to provide the surgeon with the largest number of suitable donors by minimising the number of false positive results. Postmortem serological testing considerably reduces, but does not totally suppress, the risk of contamination.[4] Apart from discrepancies between HIV infection status and serological findings that occur during the antibody negative window period,[5] false negative results cannot be totally ruled out. Even with the current enzyme immunoassays and HIV p24 antigen detection methods,[6 7] they may even be found beyond the expected window period.[8] Alternatively, testing of cadaveric tissue donor serum for blood-borne viruses is often complicated by the gross haemolysis of those samples, resulting in a high rate of serological false positive results.[9] This, in turn, may lead to the waste of material otherwise suitable for reconstructive surgery. PCR used on postmortem specimens could contribute to a better donor selection.

PCR on blood can detect HIV-1 infection before seroconversion[6]; nested PCR has recently been used to amplify the HIV proviral DNA in mononuclear cells from a persistently seronegative HIV infected patient,[8] and in postmortem mononuclear cells.[10] The rationale for performing PCR on skin is the presence of HIV proviral DNA and RNA in epidermal Langerhans' cells isolated from HIV infected individuals.[11] Moreover, infected Langerhans' cells are a potential route for the spread of HIV to normal human keratinocytes[12] and play a role in the dissemination of HIV infection.[13–16] Nevertheless, the in vivo transmission of HIV-1 through a contaminated skin graft has been suggested only once,[4] which calls into question the infectivity of infected skin.

Viral culture was performed on cadaveric skin and blood in order to assess their postmortem infectivity in HIV-1 infected patients. At the same time, nested PCR on skin and blood using gag and pol specific primers was compared to serology. Other parameters

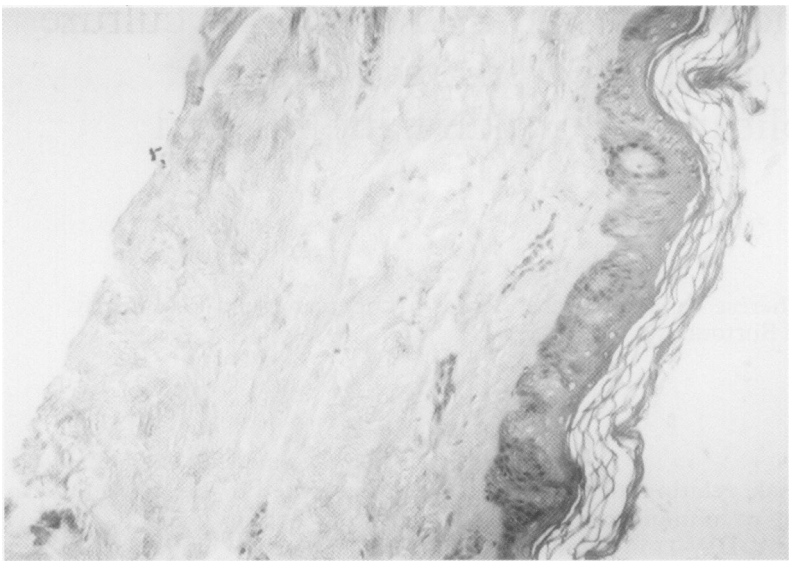

Figure 1 Paraffin section of an HIV contaminated postmortem skin specimen. The specimen was collected 24 hours after death with an electric dermatoma, fixed in Bouin's fluid and stained with haematoxylin and eosin.

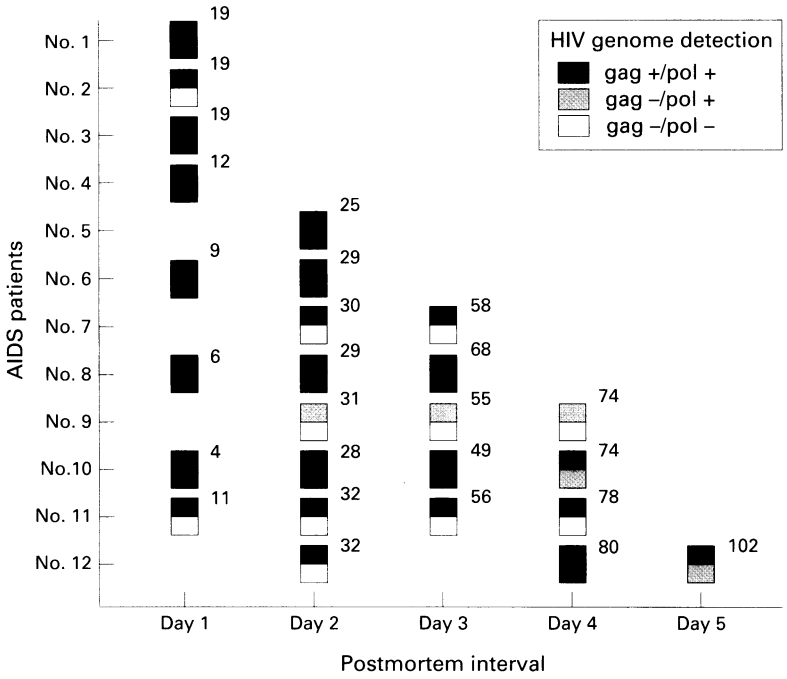

Figure 2 Results of HIV-1 detection by nested PCR in skin and blood postmortem samples. Top square, skin; bottom square, blood; numbers are postmortem interval in hours. For each skin specimen collected, PCR was performed on fresh and DMSO and glycerol cryopreserved samples. For patients 1–5, no DMSO stored sample was available. PCR results were identical for these different types of skin sample and are, therefore, represented by a single square.

including the time interval between death and sample collection and the effects of the cryopreservation procedure were also examined.

Methods

SAMPLES

After family consent, the cadavers of 12 patients with aquired immunodeficiency syndrome (AIDS) and five HIV negative controls who died at the St Luc University Hospital were included in the study. In the hour following death, each body was placed in a refrigerated vault at a temperature between 2°C and 4°C. Skin and blood specimens were collected at the same time during the first 48 hours after death from the 17 patients. For seven HIV infected cadavers, tissue and blood were collected daily until burial, except for patient 12 (day 3 missing). The postmortem interval (the time between death and sample collection) varied between four and 102 hours. After thorough skin shaving and cleaning with antiseptic and sterile paraffin, a 5–10 mm thick dermo-epidermal sheet (fig 1) was obtained from the thigh by using an electric dermatoma (Aesculap AG, Germany). Skin sheets were tested for HIV using viral culture and PCR both after collection (n = 31) and after cryopreservation (n = 57). The samples, including those from the five controls, were cryopreserved in 5% dimethylsulphoxide (DMSO) (n = 26) as well as in 30% glycerol (n = 31) and maintained in liquid nitrogen until retesting. Concurrently, 2–4 ml of blood was drawn from the jugular, subclavian, or inguinal vessels, in both ethylenediamine tetra-acetic acid containing tubes and plain glass tubes.

SAMPLE PREPARATION

Blood and skin specimens for direct testing were either prepared immediately or maintained, for a maximum of 48 hours, at 4°C until processed. Blood mononuclear cells were separated by centrifugation over Lymphopaque gradient. Skin samples were minced with a scalpel and the cells collected after enzymatic digestion (trypsin, Gibco BRL Life Technologies, Scotland). Cells were washed in phosphate buffered saline.

NESTED POLYMERASE CHAIN REATION

A nested PCR was performed using gag and pol specific primers as described previously.[17] DNA was extracted from a total of 2×10^6 mononuclear cells for blood and a range of $0.1–2 \times 10^6$ (mean 1.2×10^6) cells for the skin. After resuspension in 50 µl buffer (100 mM KCl, 10 mM Tris HCl, pH 8.3, and 2.5 mM $MgCl_2$), the cells were lysed in 50 µl lysis buffer (10 mM Tris HCl, pH 8.3, 2.5 mM $MgCl_2$, 1% Tween, and 1% NP40) with 4 µl proteinase K at 25 mg/ml. After digestion for one hour at 60°C, the enzyme was inactivated by heating for 15 minutes at 95°C. During the first amplification step, 5 µl of the DNA solution was mixed with PCR reaction buffer (final volume 50 µl). Two sets of primers, recognising a 473 base pair sequence of the gag gene and a 244 base pair sequence of the pol gene, were used separately. In the second amplification step, 1 µl of the amplified product was mixed with PCR buffer (final volume 50 µl) and primers recognising a 248 base pair sequence for the gag gene and a 128 base pair sequence for the pol gene. The HIV-1 infected cell line 8E5, containing a single copy of the HIV-1 proviral DNA, was used as a positive control.[18] Mononuclear cells from seronegative blood donors were used as negative controls.

The cells were resuspended in culture medium at a concentration of 10^6 cells/ml. Blood or skin cells (2×10^5 cells) were co-cultivated with phytohaemagglutinin (PHA) stimulated CD4 lymphocytes from HIV-1 seronegative donors in the presence of

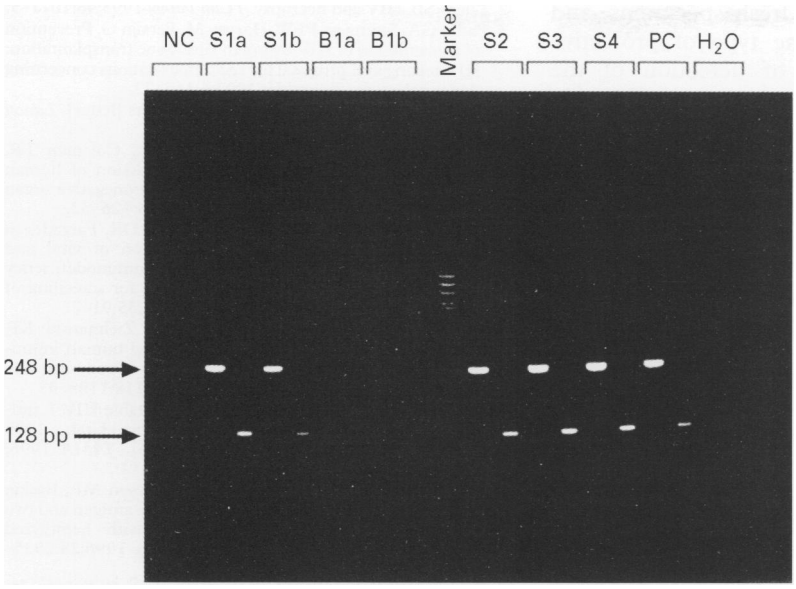

Figure 3 Illustration of the nested PCR results with blood and skin specimens: 248 base pair (bp) gag sequence; 128 bp pol sequence; Marker, molecular weight marker; NC, negative control (blood from a seronegative blood donor); S1a/S1b, skin sample from patient number 2 (analysis in duplicate); B1a/B1b, blood sample from patient number 2 (analysis in duplicate); S2/S3/S4, skin samples from patients 3, 4, and 5, respectively; PC, positive control.

5 UI/ml recombinant interleukin-2 (Biotest, Belgium) and 2 mg/ml Polybrene. The co-cultures were incubated for a period of 24 days. Twice a week the supernatants were replaced with fresh medium and once a week 5×10^4 PHA stimulated CD4 lymphocytes were added to the culture. The supernatants were tested twice a week for the presence of HIV-1 p24 antigen with a commercial enzyme immunoassay kit (Coulter). A culture was considered positive for HIV-1 if two serial supernatant samples were positive consecutively with a twofold or greater increase in reactivity.

Serum samples were assayed using Wellcozyme HIV1+2 (Murex, Wellcome, UK), Serodia HIV1/2 (Lameris, Fujirebio, Japan), Coulter HIV-1 p24 Antigen Assay (Coulter, Hialeah, USA), and confirmation tests New LAV Blot I/II (Sanofi Pasteur, Institute Pasteur, France), according to the manufacturers' instructions.

Results

In all the skin specimens (n = 73) from the infected patients, PCR detected HIV proviral DNA, regardless of the postmortem interval and the cryopreservation procedure (fig 2); gag and pol sequences were found in the serial fresh and thawed skin specimens from 11 of 12 patients (fig 3) while the pol sequence only was detected in one patient (number 9).

The number of PCR positive blood samples with respect to postmortem interval were: six of eight (day 1), four of eight (day 2), one of five (day 3), two of four (day 4), and one of one (day 5). However, two late samples (days 4 and 5) were positive only for the pol sequence while being positive for gag and pol on earlier samples. Altogether, both sequences were detected in only eight of 12 (67%) patients, corresponding to 12 of 26 (46%) blood samples (fig 3). The HIV-1 sequences were not detected in the consecutive blood samples from three different cases (patients number 7, 9, and 11) or in the sample from patient number 2. The average postmortem interval for the positive and negative results was 35 hours (range, 4–102 hours; mean (SEM), 35 (8)) and 45 hours (range, 11–78 hours; mean (SEM), 45 (7)), respectively (not statistically significant). No positive results were obtained for blood or skin specimens from the HIV negative patients and there were no false negative results with the 8E5 cell line.

Among the AIDS patients, HIV-1 was detected in viral cultures of nine of 73 skin specimens (12%) obtained from four of 12 bodies (33%) (table 1). Positive cultures were obtained from two of 26 fresh skin samples (patients 9 and 10), three of 21 DMSO samples (patients 7, 10, and 11), and four consecutive glycerol specimens collected from the same body (patient number 10). It was also detected in eight of 26 blood specimens (31%) collected from six of 12 AIDS patients (50%). All the viral cultures of blood and skin from the HIV negative patients were negative.

Anti-HIV antibodies were found in each postmortem plasma sample from HIV infected patients. No discrepant results were obtained between the assays.

Table 1 HIV-1 positive cultures and postmortem interval for the 12 AIDS patients

		Cryopreserved skin		
Patient number	*Fresh skin (n=26)*	*DMSO (n=21)*	*Glycerol (n=26)*	*Blood (n=26)*
1	–	ND	–	–
2	–	ND	–	–
3	–	ND	–	19 hours
4	–	ND	–	–
5	–	ND	–	25 hours
6	–	–	–	9, 29 hours
7	–	58 hours	–	–
8	–	–	–	6, 29 hours
9	31 hours	–	–	–
10	49 hours	28 hours	4, 28, 49, 74 hours	4 hours
11	–	78 hours	–	–
12	–	–	–	80 hours

Discussion

The persistence of HIV-1 infectivity has been reported in fresh[10 19] or frozen postmortem specimens[20] including blood, bone, brain, spleen, lung, and lymphoid tissue; this highlights the potential risk for transmission by cadaveric material. However, infectivity does not appear to be equivalent for all types of tissues: among the unprocessed specimens, fresh-frozen bone appears more infective than corneas, lyophilised soft tissue, or marrow evacuated fresh-frozen bone.[5] The transmission of HIV-1 infection through skin grafts has been hypothesised in a short case report,[4] but the risk related to the presence of HIV-1 in cadaver skin is so far unknown.[2] In the current study, positive cultures obtained with fresh, as well as glycerol and DMSO cryopreserved skin specimens, clearly demonstrate the potential infectivity of this material.

Using nested PCR, detection of the HIV sequences in skin specimens was successful on every skin sample from HIV infected patients, even samples collected several days after death. Results using cryopreserved skin were identical

to those obtained from fresh specimens, and were independent of the type of protective agent used. The lack of detection of the gag genomic sequence in the consecutive skin samples from one patient illustrates the need for amplification of more than one HIV genomic sequence to increase PCR sensitivity. Whether HIV-1 can be detected by PCR in skin at an early stage of infection remains to be determined. Taking into account the contribution of Langerhans' cells in HIV dissemination,[11-16] this point deserves further evaluation.

In contrast to skin PCR, false negative results were obtained by PCR in about 50% of all the blood specimens including some of those collected during the first 24 hours, the usual time interval for skin graft harvesting. While blood samples remained consistently negative in some patients, discordant results were observed on successive samples in others. Several factors including PCR inhibitors, variability relating to the degree of cellular lysis and site of blood collection might interfere with PCR results.

Anti-HIV antibodies were found in the plasma of all our infected patients, which confirms that test kits remain undoubtedly the easiest postmortem detection tool in blood,[21-23] but it should be borne in mind that their specificity is not guaranteed when applied to postmortem blood of HIV negative patients and may lead to a high rate of false positive results.[9] The US Food and Drug Administration does not require kit manufacturers to submit data showing that HIV-1 antigen and antibody detection kits produce accurate results with samples from cadaveric donors.[24]

In conclusion, while blood screening remains the key safety procedure used by skin banks to detect HIV infected patients, data suggest that nested PCR on postmortem skin samples can detect HIV more reliably than on blood. This study also extends previous data on HIV and necropsy.[2] In addition, it underscores the need for caution during the handling of skin postmortem tissue from HIV infected cadavers and confirms the potential risk related to accidental allografting of HIV contaminated skin. Further studies are now needed to evaluate the potential impact in donor selection.

We thank Dr B Delaere and P Goubau for their critical readings and helpful suggestions, the nursing staffs of the Burn Unit Center of the Queen Astrid Military Hospital for assistance, and the Medical Staff section of the Belgian Army for providing technical facilities.

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