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HIV infection reduces skin graft survival in burn injuries: a prospective study☆

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KEYWORDS
Burns; HIV infection; Split skin graft; Early excision

Summary Impaired survival of skin grafts has been noted in human immunodeficiency virus (HIV) infected patients, but the reason is not known. Alterations in inflammatory response, which might be recorded as an imbalance in cytokine production, have been implicated.

The aim of this study was to determine the impact of HIV infection in patients with burn injuries by comparison of split skin graft survival, T lymphocyte count and cytokine levels in HIV-infected and non HIV-infected patients in relation to healthy and HIV-infected nonburnt volunteers.

Fifty-four patients with deep dermal burns were included. Fifteen patients' were HIV-infected. Thirteen healthy and 15 HIV-infected, volunteers were recruited as controls.

The burnt surface area was traced on a transparent plastic sheet and converted to area. Graft survival on day of discharge/regraft for non HIV-infected patients was 69%, and in HIV-infected 22%, ($p < 0.05$). The median length of hospital stay for early excision among non HIV-infected patients was 21 (12–53) days and for HIV-infected, 41 days ($p < 0.05$).

Serum protein levels in HIV-infected patients were elevated compared to non HIV-infected patients ($p < 0.05$). CD4+ lymphocytes were depressed in HIV-infected volunteers and HIV-infected burn patients compared to healthy volunteers ($p < 0.05$). CD8+ lymphocytes were elevated in HIV-infected volunteers compared to non HIV-infected burn patients. Pro-inflammatory cytokine levels of Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interferon-gama (IFN-γ) and tumour necrosis factor alpha (TNF-α) were depressed in HIV-infected volunteers compared to healthy volunteers and non HIV-infected burn patients. The pro-inflammatory cytokine IFN-γ did not increase after burn injury in HIV-infected burns patients as did IL-2, IL-6 and TNF-α ($p < 0.05$). Anti-inflammatory cytokine levels of IL-4 were elevated in HIV-infected volunteers compared to healthy volunteers and burn patients ($p < 0.05$).

Conclusion: Graft survival after split skin grafting of burn wounds in HIV-infected patients is impaired and hospital stay is prolonged. HIV infection result in immune dysregulation, which might be related to impaired skin graft survival.

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Introduction

In sub-Saharan Africa, the prevalence of the human immunodeficiency virus (HIV) infection in 1999 in the adult population of the worst affected countries; Botswana, Swaziland and Zimbabwe was 39, 35 and 25%, respectively.[1,2] In Zimbabwe, HIV infection has reduced life expectancy to 40 years when it should have been 69 years and furthermore resulted in a crude death rate of 21 per 1000 compared to five per 1000 without the HIV pandemic.[2] HIV infection has resulted in major changes of symptoms, signs and pathology of certain common diseases.[3-5]

In immuno-compromised patients the healing of perineal wounds and laparotomy incisions has been found to be delayed.[6-8] Impaired survival of split skin grafts has been noted in HIV-infected patients, but the reason is not known.[9] Alterations in inflammatory response as mediated by cytokines may be implicated.[10,11] The preferred method of burn wound treatment is early excision and split skin grafting, which has been shown to reduce morbidity, mortality, duration of hospital stay and costs.[12-18] The healing of skin grafts in burn wounds in HIV-infected patients has to our knowledge not been evaluated in prospective studies.

The aim of this study was to determine the impact of HIV infection in patients with burn injuries by comparing split skin graft survival in HIV-infected and non HIV-infected patients after early excision and grafting or delayed split skin grafting and to compare T lymphocyte and cytokine levels in HIV-infected, and non HIV-infected burns patients, and in HIV-infected and non HIV-infected volunteers.

The local ethical committee approved the study.

Material

Fifty-four patients, 18 children (0-15) years, and 36 adult patients, with flame burns and scalds between 10 and 20% of the Total Body Surface Area (TBSA) were included. Electrical and chemical burns were excluded. Fifteen patients were HIV-infected. The median age of the HIV-infected patients was 27 (15-45) years and of the non HIV-infected 22 (3-51) years. Twenty-nine patients had early excision and split skin grafting, nine of these were HIV-infected. Twenty-five patients had delayed split skin grafting; six of these were HIV-infected (Fig.1).

Thirteen healthy volunteers, median age 22 (20-42) years served as nonburnt, non HIV-infected controls. Fifteen HIV-infected nonburnt volunteers median age 25(19-43) years served as nonburnt HIV-infected controls.

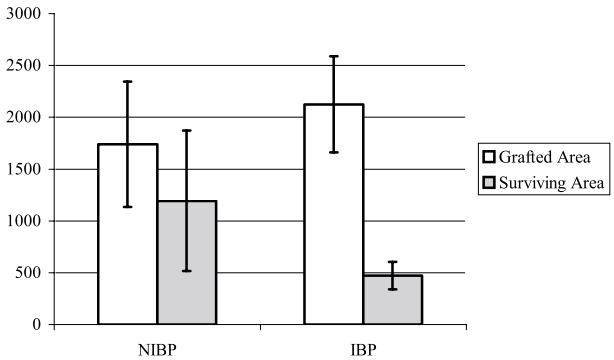

Fig. 1 Skin graft survival in burns patients. Mean (SD) (cm^2). NIBP, non HIV-infected burn patients; IBP, HIV-infected burn patients. Area of surviving graft is less in IBP than in NIBP ($p < 0.05$).

Methods

Early excision and split skin grafting was performed on 29 patients. Twenty-five patients had delayed split skin grafting. Before skin grafting, the burnt surface area was traced on a transparent plastic sheet. The tracings were cut out and weighed on an electronic balance. The weight of the cut out plastic sheet was converted to area. In 54 patients blood samples were taken the day of operation and analysed for HIV infection using the enzyme-linked immunosorbent assay (ELISA), (ENZYGNOST, Marburg, Germany). Blood was taken and analysed for serum proteins, T lymphocyte count of CD4 + and CD8 + cells as well as serum cytokine levels of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α). The number of patients analysed are indicated in the tables. Serum cytokines were analysed using ELISA kits. (PHARMIGEN, San Jose, California, USA). Graft survival as a measure of wound healing was calculated by, tracing the surviving graft on a transparent plastic sheet at the time of discharge or regrafting. A specially trained nurse counselled all HIV-infected patients. Blood samples from healthy volunteers and HIV-infected volunteers were taken for analysis of the same parameters as in burnt patients.

Statistical methods

The statistical package Epi-Info was used to calculate means, standard deviations, medians and ranges. The Mann-Whitney and the Chi-squared

tests were used to test the significances of the differences between various parameters. *P*-values <0.05 were considered significant.

Results

Twenty-eight percent of all burn patients were HIV-infected. Of the adult population 14/36 (39%) were HIV-infected (Table 1).

For the non HIV-infected patients, the mean area of the surviving grafts on the day of discharge or regraft was 69% of the graft on the day of operation (1194 ± 677 cm^2 compared to 1739 ± 604 cm^2). In HIV-infected patients the corresponding figure was 22%, (472 ± 132 cm^2 compared to 2125 ± 463 cm^2), giving $p < 0.05$.

The median length of hospital stay for early excision in 20 non HIV-infected patients was 21 (12–53) days and for nine HIV-infected patients 41 (25–73) days ($p < 0.05$). The median length of hospital stay for delayed skin grafting in 19 non HIV-infected patients was 39 (29–123) days, and in six HIV-infected patients it was 48 (35–86) days (n.s.).

Eight of 39 non HIV-infected patients, were regrafted. Of these three developed hypertrophic scars. Five of 15 HIV-infected patients were regrafted. One had hypertrophic scarring. One HIV-infected female patient died of pneumonia. Bacterial growth in wound swabs was observed in six of 39 non HIV-infected patients. One out of 15 HIV-infected patients had bacterial growth on wound swabs. The most common isolates were *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

In patients with clinical signs of sepsis, two of 17 non HIV-infected patients had bacterial growth as verified by blood culture and in the HIV-infected patients, one of six.

Haematological parameters are presented in Table 2.

In healthy volunteers, the levels of haemoglobin and haematocrit were higher than in non HIV-infected burns patients, HIV-infected volunteers and HIV-infected burn patients ($p < 0.05$). Leucocyte levels were elevated in burn patients compared to healthy volunteers ($p < 0.05$). In non HIV-infected burn patients, platelets were elevated compared to HIV-infected burns patients and both healthy and HIV-infected volunteers ($p < 0.05$). In HIV-infected burn patients; levels of serum proteins were elevated compared to non HIV-infected burns patients and healthy volunteers ($p < 0.05$).

Counts of T lymphocytes are presented in Table 3.

In healthy volunteers the count of CD4+ lymphocytes was higher than in HIV-infected burn patients and HIV-infected volunteers ($p < 0.05$). Between healthy volunteers and noninfected burns patients, the difference in CD4+ count approached significance ($p < 0.06$) (Fig. 2). In HIV-infected volunteers the counts of CD8+ lymphocytes were elevated compared to non HIV-infected burn patients and healthy volunteers ($p < 0.05$).

The ratio of CD4+/CD8+ lymphocytes in HIV-infected burn patients and HIV-infected volunteers was less than half of that in non HIV-infected patients and healthy volunteers. The ratio was also reversed to below one in non HIV-infected burns patients.

Serum cytokine levels are presented in Table 4.

In HIV-infected volunteers the pro-inflammatory cytokines IL-2, IL-6 and TNF-α were depressed compared to healthy volunteers, non HIV-infected burn and HIV-infected burn patients ($p < 0.05$). In both HIV-infected volunteers and HIV-infected burns patients IFN-γ was depressed compared to healthy volunteers and non HIV-infected burns patients ($p < 0.05$). In HIV-infected volunteers anti-inflammatory cytokine IL-4 was elevated compared to healthy volunteers, non HIV-infected burn patients, and HIV-infected burn patients ($p < 0.05$).

Table 1 Number of non HIV-infected and HIV-infected burns patients in relation to sex

	Adults		Children		Total
	Male	Female	Male	Female	
NonHIV-infected	9	13	6	11	39
HIV-infected	9	5	1	0	15
Total	18	18	7	11	54

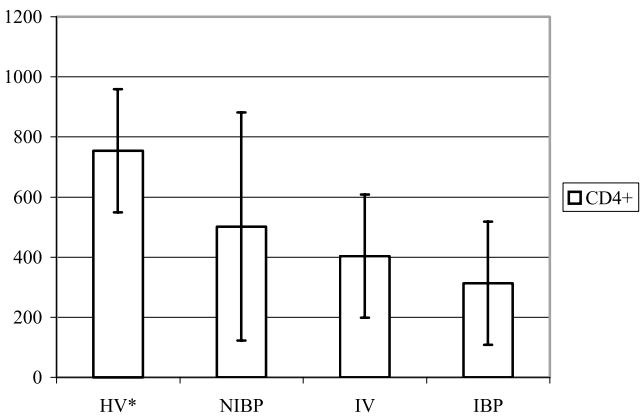

Fig. 2 Number of CD4 positive lymphocytes ($\times 10^3$). Mean (SD). HV, healthy volunteers; NIBP, non HIV-infected burn patients; IV, HIV-infected volunteers; IBP, HIV-infected burn patients. *Indicates CD4+ lymphocytes are higher in HV compared to IV and IBP ($p < 0.05$).

Table 2 Haematological parameters, mean (SD)

	HV ($n = 13$)	NIBP ($n = 39$)	IV ($n = 15$)	IBP ($n = 15$)
Haemoglobin g%	15 (2)[a]	13 (2)	11 (2)	12 (2)
Haematocrit %	48 (4)[a]	34 (7)	39 (6)	36 (6)
Leucocytes $\times 10^3$	5 (2)	10 (4)[b]	6 (1)	8 (4)[b]
Platelets $\times 10^3$	194 (33)	352 (137)[c]	251 (67)	263 (72)
Serum protein g%	60 (10)	64 (11)	74 (6)	82 (47)[d]

HV, healthy volunteers; NIBP, non HIV-infected burn patients; IV, HIV-infected volunteers; IBP, HIV-infected burn patients.
[a] Indicates differences compared to NIBP, IV, and IBP ($p < 0.05$).
[b] Indicates elevated levels compared to HV ($p < 0.05$).
[c] Indicates elevated levels compared to HV, IV and IBP ($p < 0.05$).
[d] Indicates elevated levels compared to HV and NIBP ($p < 0.05$).

Table 3 Lymphocyte count $\times 10^3$, mean (SD) and CD4/CD8 ratio

Lymphocytes	HV ($n = 13$)	NIBP ($n = 12$)	IV ($n = 15$)	IBP ($n = 15$)
CD4 +	754(205)[a]	505(380)	403(205)	313(205)
CD8 +	677(226)	594(522)	1059(640)[b]	877(354)
CD4/CD8	1.1	0.9	0.4	0.4

HV, healthy volunteers; NIBP, non HIV-infected burn patients; IV, HIV-infected volunteers; IBP, HIV-infected burn patient.
[a] Indicates differences compared to IV and IBP ($p < 0.05$).
[b] Indicates differences compared to HV and NIBP ($p < 0.05$).

Table 4 Serum cytokine levels, mean (SD) (pg/L)

Cytokines (pg/L)	HV ($n = 13$)	NIBP ($n = 12$)	IV ($n = 14$)	IBP ($n = 12$)
IL-2 (pro-)	58 (24)	24 (27)	7 (6)[a]	19 (24)
IL-4 (anti-)	43 (71)	156 (164)	403 (205)[b]	197 (145)
IL-6 (pro-)	106 (145)	89 (126)	4 (3)[a]	73 (103)
TNF-α (pro-)	130 (160)	127 (90)	10 (4)[a]	108 (94)
IFN-γ (pro-)	68 (92)	61 (65)	4 (3)[c]	18 (11)[c]

HV, healthy volunteers; NIBP, non HIV-infected burn patients; IV, HIV-infected volunteers; IBP, HIV-infected burn patients. (pro, pro-inflammatory; anti, anti-inflammatory).
[a] Indicates reduced levels compared to HV, NIBP and IBP ($p < 0.05$).
[b] Indicates elevated levels compared to HV, NIBP and IBP ($p < 0.05$).
[c] Indicates reduced levels compared to HV and NIBP.

Discussion

Thirty-nine percent (14/36) of the adult burn patients were HIV-infected. This hospital frequency of HIV infection is higher than the national HIV prevalence, which is between 25 and 33% depending upon location.[19]

According to the UNAIDS report on the epidemiology of HIV in Zimbabwe, the prevalence of HIV infection in the age group 15–49 years was 25% at the end of 1999. However, in urban areas the estimated prevalence was more than 30%.[1]

One male child, 15 years old, was HIV-infected, probably through heterosexual transmission, the most prevalent mode of transmission in Africa.[20]

The frequency of HIV infection among patients admitted to the Harare burn unit of 39% is similar to a recent study in Harare, where 34% of adult patients admitted to one of the major teaching hospitals for emergency abdominal surgery operations were HIV-infected.[21] In a South African study of general surgery patients, 1/3 were HIV-infected with low CD4+ T lymphocyte counts.[22] Infection with HIV in general surgery patients are associated with considerable morbidity and mortality, patients generally dying from HIV related infections.[21] However, increased mortality was not recorded in a recent report of burn injuries from Cape Town, South Africa. Morbidity in the form of graft survival was not studied.[23]

In this report graft survival among the HIV-infected patients was only 22%, compared to 69%, in non HIV-infected patients. The reason for the poor graft survival between HIV-infected patients is not obvious. The most frequent cause of postoperative skin graft failure in burn patients is bacterial infection especially with beta haemolytic streptococci and *Pseudomonas aeruginosa*.[24–26] In this study, there was no difference in infection rate as measured by wound swab cultures and blood cultures between infected and non HIV-infected patients as also recorded in another study.[27] Despite similar rates of bacterial growth in cultures, bacterial infection cannot be completely excluded as a contributing factor of the graft failure noted. However, the HIV pandemic has changed the pattern of postoperative complications not necessarily related to infection, therefore, other factors must be considered.[3,4,21]

The median duration of hospital stay for non HIV-infected patients who had early excision and skin grafting was 21 days compared to 41 days in the nine HIV-infected patients who had the same type of surgery. This difference is related to the high rate of graft failure and frequent regrafting between the HIV-infected patients. Early excision and skin grafting has a number of advantages including reduction of morbidity and mortality, length of hospital stay and costs.[9,11,26,27]

Early excision of the burnt skin, removes the eschar with the lipoprotein complex (LPC) thereby limiting the inflammatory response.[28] The LPC elicits production of pro-inflammatory cytokines such as IL-2, IL-6, TNF-α and IFN-γ as an immunological response to T lymphocyte activation.[29] Both burn trauma and HIV infection lead to changes in the number of immune competent cells including T lymphocytes with subsequent modulation of release of different cytokines.[30]

In the HIV-infected burn patients the mean count of CD4+ cells was reduced and CD8+ lymphocyte count was elevated. The CD4/CD8 ratio was reversed (less than one), not only in HIV-infected volunteers and HIV-infected burn patients, but also in non HIV-infected burns patients (Table 3). This is suggestive of an additive effect of burn injury and HIV infection on immuno-suppression (Fig. 2).

The HIV destroys CD4+ cells through several mechanisms including syncytium formation, cytotoxicity, and apoptosis.[31,32] The body responds immunologically by increasing the production of CD8+ lymphocytes, which also kill HIV, infected CD4+ lymphocytes.[33] It is known that the HIV and the CD8+ lymphocytes kill the CD4+ cells and reduce their number in circulation.[34] The additive immunosuppressive effect of burn injury and HIV infection might explain the pronounced depletion of CD4+ lymphocytes in HIV-infected burn patients (Fig. 2, Table 3).

There was no difference in haematological parameters between HIV-infected and non HIV-infected burns patients except for elevation of serum protein levels in HIV-infected burn patients, and elevation of platelets in non HIV-infected burns patients. The elevated serum protein level might be explained by an abnormal activation of B lymphocytes producing immunoglobulins leading to hypergammaglobulinemia.[35] The elevation of immunoglobulin, low CD4+ and high CD8+ lymphocyte counts and relatively low platelet counts suggests immuno-suppression in the HIV-infected burns patients.

In HIV-infected volunteers the pro-inflammatory cytokines IL-2, IL-6, TNF-α and IFN-γ are depressed as compared to healthy volunteers. By contrast in HIV-infected patients with burn injuries, these cytokines were at the same level as in non HIV-infected burn patients except for IFN-γ which remained depressed after burn injury. This suggests that HIV-infected patients respond to burn injury with sufficient production of IL-2, IL-6, and TNF-α, but lack this response in respect of IFN-γ.

In HIV-infected volunteers, the anti-inflammatory cytokine IL-4 was elevated compared with healthy volunteers. It is known that IL-4 is elevated in established HIV infection, but falls when AIDS develops.[36] However, in HIV-infected patients with burn injuries, the anti-inflammatory cytokine IL-4 is suppressed to the same level as in noninfected burn patients and the anti-inflammatory imbalance in respect of IL-4 seems restored.

Only IFN-γ among the investigated cytokines, remained at the same level in both HIV-infected volunteers and in non HIV-infected burn patients (Table 4). The poor response to burn injury by HIV-infected patients as reflected by low levels of IFN-γ is probably related to reduced production by macrophages. Macrophages are stimulated to produce cytokines by CD4+ cells but these cells were decreased in HIV-infected volunteers and even more so in HIV-infected burns patients (Table 3, Fig. 2).

Whether inadequate cytokine production by macrophages in HIV-infected burns patients is contributory to poor split skin graft survival or not can at present only be regarded as a hypothesis.

In conclusion, split skin graft survival of burn wounds in HIV-infected patients is impaired and hospital stay is prolonged.

HIV infection result in immune dysregulation, which might be related to impaired skin graft survival.

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