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Posttraumatic Natural Killer Cell Decrease is Associated with Septic Complications

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ABSTRACT

Background: There has been paucity in prospective studies investigating trauma-induced changes in the cellular immunity of HIV-seropositive patients and their impact on the clinical outcome after trauma surgery. The role of natural killer (NK) cells especially has not yet been fully elucidated, and the function of this lymphocyte subtype in the immune defense after trauma is still under debate.

Methods: This prospective study included patients requiring surgery for abdominal gunshot wounds. A blood specimen was obtained on admission, 48 hours after the index operation and, in case of damage control surgery, 48 hours after the first planned second look operation. The quantity and changes of T-, B- and NK cells were analyzed via flow cytometry to investigate whether these numbers had an impact on the postoperative outcome.

Results: A total of 62 patients were recruited in the analysis of which 38 were HIV-negative and 24 HIV-seropositive. After surgery, HIV-negative patients had a more severe decrease of their CD4+ T cells compared to the HIV-seropositive patients. Trauma resulted in a severe decrease of NK cells irrespective of the HIV-serostatus. Patients with more extensive NK cell drop had a significantly higher postoperative complication rate.

Conclusions: Our data support the association of trauma-induced NK cell decrease with a subsequent significantly higher rate of septic and surgical complications and suggest that these immune cells might play an important role in antibacterial immunity. Strengthen-

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ing the NK cell function or limiting their decrease in the postoperative course might be of therapeutic value in severely injured trauma patients.

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Introduction

While total trauma-related mortality has decreased in the last decade, the rate of sepsis-related mortality in trauma has remained unchanged. This highlights the ongoing, unresolved problem of the trauma-induced impairment of the immune system [1]. There is solid evidence that persistent posttraumatic lymphopenia is associated with a higher incidence of multi organ failure (MOF) as well as with increased mortality [2,3].

Almost 15% of circulating lymphocytes are natural killer (NK) cells which play a central role in the innate immune response and function as the central link to the adaptive immune system [4]. The role of NK cells in trauma patients, its changes and impact on the postoperative course and septic complications, in particular, have not been fully elucidated. Specifically, studies that have investigated the trauma-induced NK cell changes report inconclusive or contradictory results. Where some report an early decrease of NK cells after injury [5], others have described only functional alterations of NK cells without quantitative changes [6,7].

Through their cytokine-driven interaction with other lymphocytes, NK cells boost the differentiation of CD4+ T cells [8]. The latter, together with other inherent activities of NK cells, such as cytotoxicity and their ability to destroy virus-infected cells, render NK cells an important pillar in the immune response to infections, including HIV.

Persistently high prevalence of HIV-infection amongst South African trauma patients [9] substantiates the importance to better understand the posttraumatic dynamics and differences in cellular immunity within this patient group.

To the best of our knowledge, no study to date has explored whether a different NK cell dynamic profile is present in HIV-seropositive compared to HIV-negative trauma patients, or whether quantitative changes of these cells after penetrating abdominal injury influence the clinical outcome.

Our study aimed to test our hypotheses that trauma induces a different cellular immune response in the presence of HIV, and that trauma leads to a NK cell decrease which, in turn, results in more septic complications.

Material and Methods

This prospective study recruited HIV-seropositive and -negative adult patients from December 2018 to March 2020 presenting to the Chris Hani Baragwanath Academic Hospital (CHBAH) Trauma Unit in Johannesburg, South Africa, who required surgery for abdominal gunshot injuries with bowel involvement. In particular, this injury pattern was selected to avoid bias from different injury mechanisms and, because it results in a perforated hollow viscus, this surgery is classified as dirty or infected, hence predisposes to create septic abdominal foci [10]. Exclusion criteria were patients with uncontrolled diabetes mellitus (A1C>7%), active tuberculosis infection, a history of prolonged steroid use, pre-existing organ failure, and demise within 72 hours of admission. In addition, 11 patients who underwent small bowel resection for non-traumatic indications, without physiological derangements, signs of sepsis or immune compromise at the time of surgery, were recruited as the control group.

The protocol was approved by the Human Research Ethics Committee of the University of the Witwatersrand (clearance number M180914). Informed consent was obtained by the patient him/herself or, if the patient had a decreased level of consciousness on admission, by the patient's next of kin.

In all study patients a blood specimen for assessment of HIV-status, NK cells, CD3+ T-, CD4+ T-, CD8+ T-, and B-lymphocytes was sampled on admission (A-blood), 48 hours after the index operation (P1-blood) and, in cases of damage control surgery, 48 hours after the first planned second look operation (P2-blood). In all HIV-seropositive patients, the viral load, treatment status, treatment duration, drug combinations and presence of AIDS (acquired immunodeficiency syndrome)-defining conditions were also documented. The entirety of the sustained intra-abdominal injuries was graded according to the penetrating abdominal trauma index (PATI) [11].

Postoperative care was conducted according to general surgical standards and not dictated by the patient's participation in the study. The follow-up of the patients was restricted to the in-hospital course and a weekly review in our trauma clinic for a period of one month after discharge from the hospital. Postoperative outcome was measured according the number and nature of septic complications, as well as the postoperative surgical complications classified according to the Clavien-Dindo classification [12], categorizing grades 1-2 as minor, and grades 3-5 were as major complications. The latter division was based on grade 3 complications and higher needing invasive interventions, either surgically, endoscopically or radiologically. The reported septic complications included: superficial and deep surgical site infections, septicaemia with positive blood cultures, fungemia, pneumonia, septic intraabdominal collections (diagnosed radiologically or during relook), and urinary tract infections.

Laboratory investigations of blood

The whole blood was processed and analyzed via flow cytometry by the Division of Histopathology in the National Health Laboratory Service (NHLS) at CHBAH as follows:

Single platform CD3+ total T-lymphocytes, CD3+/CD4+ Helper T-lymphocytes, CD3+/CD8+ Suppressor T-lymphocytes, CD19+ B-lymphocytes and CD3-/CD56+ NK lymphocytes were prepared using the Immunoprep lyse-no-wash method and Cyto-stat multi-color antibody cocktails

as follows: CD45-FITC/CD4-PE/CD8-ECD/CD3-PC5 and CD45-FITC/CD56-PE/CD19-ECD/CD3-PC5 (Beckman Coulter Life Sciences Inc., Indianapolis, USA) [13]. Post preparation, Beckman Flow Count beads were added and samples acquired and analyzed on a Beckman Navios flow cytometer (Beckman Coulter Ireland, Inc., Lismeehan).

Statistical analysis

The Chi-squared test or Fisher's exact test was used, where appropriate, to assess the relationships between categorical variables according to HIV-status. Within-group tests for paired continuous data were carried out using the paired t-test and, where the data did not meet the assumptions of this test, a non-parametric alternative, the Wilcoxon matched pairs test, was used. The relationship between two continuous variables was assessed by Pearson's correlation coefficient or, where indicated, the Spearman's rank correlation coefficient was used.

To determine differences or percentage change variables for paired or repeated measurements of continuous data, the one-sample t-test was applied. Sample size calculation was carried out in G*Power [14]. To test the first hypothesis that trauma induces a different cellular immune response in the presence of HIV, a minimum of 15 patients were required for determination of a large size effect. To test the second hypothesis that trauma leads to a decrease of NK cells with subsequently more septic complications, a minimum of 54 patients were required.

Data analysis was carried out using STATA vs 15.1 for Windows. A 5% significance level was used.

Results

Patient recruitment, demographics and perioperative factors

During the study period 167 patients required a laparotomy for abdominal gunshot wounds of which 93 patients were excluded because they had no small bowel injuries and 12 due to demise within 72 hours of admission. This resulted in a recruited group of 62 patients of which 61.3% were HIV-negative and 38.7% were HIV-seropositive. The study population demographics, injury severity, other relevant perioperative factors, as well as the injured organs are shown in Table 1; table and study population previously described in [15]. Except for HIV-seronegative patients being younger ($P = 0.013$), no significant differences were seen for these variables when grouped according to HIV-serostatus.

Of the 24 HIV-seropositive patients, 12 patients were newly diagnosed, while 12 patients knew their HIV-status. Of those who knew their HIV-status, 83% ($n = 10$) were taking ARVs (Antiretrovirals). Median treatment duration (IQR) of the patients on ARVs was 3 (3-9) years, median viral load (IQR) was 8,010 (368-64,450) copies/ml which is equivalent to a median (IQR) of 3.9 (2.4-4.8) Log copies/ml.

Postoperative changes in lymphocytic quantity

Absolute numbers and decrease of CD3+ T cells, CD4+ cells, CD8+ cells, B-cells and NK cells were analyzed via flow cytometry and are presented in Table 2 and 3. For the control patients, blood samples were only taken on admission and 48 hours postoperatively. Blood results on admission reflected the common HIV-induced changes with a significantly lower CD4+ T cell count ($P = 0.0001$) and CD4/CD8 ratio ($P = 0.0001$) in the HIV-seropositive patients compared to the HIV-negative patients. The median NK cell count was significantly higher in the HIV-negative compared to the HIV-seropositive patients ($P = 0.037$).

In a subgroup analysis of the HIV-seropositive patients, no significant difference in the CD4+ T cell count between HIV-treatment naïve and patients on ARVs was detected on admission (HIV naïve, 320 (IQR 196-440) vs. HIV treatment, 390 (IQR 238-482), $P = 0.58$; data not shown).

Between A and P2 blood, the HIV-seronegative patients had a more significant decrease in CD4+ T cells than the HIV-seropositive patients (HIV-seronegative P=0.0005 vs. HIV-seropositive P=0.0069). NK cell decrease was extensive, independent of the HIV-serostatus and resulted nearly in a depletion of this lymphocyte subtype in the P2-blood (Table 3). Lymphocyte numbers differ in Table 2 and 3 as only study patients with a full set of A, P1, and P2-blood values were included in Table 3.

It was scrutinized whether there is an association between the postoperative NK-cell and CD8+ T cells changes which showed a significant, positive correlation between the NK-cell decrease and the CD8+ T cell decrease (rho = 0.547; $P < 0.0001$, data not shown). The B-lymphocytes were the only lymphocyte subtype that showed non-significant changes of all examined immune cells in study and control group during the postoperative course.

Perioperative clinical factors and quantitative changes in circulating immune cells

To determine whether any perioperative factors influence the quantitative changes of the circulating immune cells between index and relook operations, we investigated the impact of the following clinical variables: PATI, time from index operation to planned second look operation, duration of inotropic requirement, pH and lactate on admission, hypotension on admission, and blood transfusion requirements. Of these perioperative factors, only the duration of inotropes had a significant impact on the quantities of circulating immune cells. Specifically, patients who required inotropic support for more than 48 hours had a significantly lower decrease of CD3+ T cells and CD8+ T cells, as well as B-cells. The increased transfusion requirement did not result in any significant quantitative lymphocyte changes, i.e., the dilution effect of the transfused blood did not play any notable role overall.

HIV status, immune cells and postoperative outcome

In the overall analysis of the study group, including all HIV-seronegative and HIV-seropositive patients, septic complications were significantly associated with a lower median CD4+ T cell and B cell count, and a lower CD4/CD8 ratio on admission (Table 4). The subgroup analysis in the HIV-seropositive and HIV-seronegative patients did not reveal any significant

Table 1 – Demographics, perioperative factors and injured organs of study patients according to HIV-serostatus.

Variable	HIV-seronegative patients ($n = 38$)	HIV-seropositive patients (n=24)	P-value
Age, years	31 (25-40)	41 (32-47)	0.013
Gender			0.64
Male	36 (95%)	22 (98%)	
Female	2 (5%)	2 (2%)	
Time injury-to-operation (h)	4 (3-6)	5 (4-6)	0.51
PATI	27 (17-33)	27 (16-32)	0.79
pH, on admission	7,34 (7,26-7,43)	7,33 (7,26-7,36)	0.32
Lactate (mmol/L), on admission	4,5 (3,2-6,6)	4,7 (3,1-5,4)	0.82
Bicarb (mmol/L), on admission	21,7 (16,3-23,2)	21,9 (17,8-23,8)	0.59
BE (mmol/L), on admission	-4,1 (-8,8 to -1,0)	-4,0 (-7,4 to -1,6)	0.85
Fecal contamination			>0.99
Mild	5 (13%)	3 (13%)	
Moderate	10 (26%)	6 (25%)	
Severe	23 (61%)	15 (63%)	
Colonic injury	25 (66%)	13 (54%)	0.43
Liver injury	3 (8%)	4 (17%)	0.42
Stomach injury	5 (13%)	5 (21%)	0.49
Vascular injury	11 (29%)	4 (17%)	0.37
Involvement of other organs	10 (26%)	9 (38%)	0.40
Inotrope requirement	23 (61%)	15 (63%)	>0.99
Inotrope duration (h)	41 (7-66)	50 (24-96)	0.21
Hypotension (SBP<90mmHg), on admission	10 (26%)	7 (29%)	>0.99
Blood products			
RBC, units			0.45
0	15 (39%)	5 (21%)	
1-2	10 (26%)	10 (42%)	
3-4	9 (24%)	6 (25%)	
5 or more	4 (11%)	3 (13%)	
FFP, units			>0.99
0	18 (47%)	11 (46%)	
1-2	9 (24%)	5 (21%)	
3 or more	11 (29%)	9 (38%)	
Platelets, units			>0.99
0	31 (82%)	20 (83%)	
1 or more	7 (18%)	4 (17%)	
Damage control surgery	19 (50%)	12 (50%)	>0.99

Continuous variables presented as median (interquartile range [IQR]). Categorical variables expressed as absolute and relative frequencies.
Abbreviations: RBC = red blood cell; FFP = fresh frozen plasma; SBP = systolic blood pressure.

associations between immune cell quantity on admission and postoperative septic complications.

In the postoperative follow-up, significantly more patients with septic complications were observed in the HIV-seropositive group compared to the HIV-negative group (HIV+: 92% vs. HIV-: 53%; $P = 0.002$). Similarly, surgical site infections were significantly more common in HIV-seropositive group (HIV+: 86% vs. HIV-: 46%; $P = 0.005$). Even though the percentage of anastomotic leaks was twice as high as that of the HIV-negative patients, this did not reach statistical significance (HIV+: 38% vs. HIV-: 19%; $P = 0.14$). Organ support requirements and ICU-length of stay (LOS) did not show marked differences according to HIV-serostatus, and where the percentage of in-hospital mortality was almost double in the HIV-seropositive patients, this did not reach statistical significance (HIV+: 38% vs. HIV-: 18%; $P = 0.14$; data not shown).

To document whether patients with a more pronounced immune cell decrease within the first 48 hours are more prone to septic and surgical complications, we categorized the study patients in two groups, one group with an extensive decrease of 50% or more, the other with a decrease of less than 50%. Septic and surgical postoperative complications were also categorized as binary variables and results of these analyses are shown in Table 5. Notably, a NK cell decreases of 50% or

Table 2 – Quantity of lymphocytes at admission (A-blood), 48 hours after the index operation (P1-blood) and 48 hours after the first planned second look operation (P2-blood) according to HIV-serostatus.

Quantity, (cells/μL)	Control group ($n = 11$)	HIV-seronegative patients ($n = 38$)	HIV-seropositive patients ($n = 24$)	P-value for overall difference between groups
CD3+				
A-blood	1999 (1609-2550)	1425 (787-2257)	1187 (646-1813)	0.058
P1-blood	1058 (497-1716)[a]	533 (420-740)[b]	578 (383-777)[b]	0.029
P2-blood		317 (263-444)	331 (274-594)	0.47
CD4+				
A-blood	1216 (892-1340)[a]	816 (481-1151)[a]	338 (204-471)[b]	0.0001
P1-blood	582 (335-794)[a]	304 (235-408)[b]	164 (106-288)[c]	0.0001
P2-blood		180 (131-276)	118 (80-183)	0.039
CD8+				
A-blood	793 (618-1214)	471 (288-907)	571 (321-1298)	0.13
P1-blood	424 (172-658)[a]	186 (120-304)[b]	334 (247-525)[a]	0.0005
P2-blood		121 (77-172)	198 (149-450)	0.012
B-cell				
A-blood	258 (140-471)[a]	206 (132-335)[a]	120 (79-179)[b]	0.0029
P1-blood	199 (117-281)[a]	171 (132-244)[a]	110 (61-155)[b]	0.012
P2-blood		139 (101-264)	91 (43-125)	0.021
NK-cells				
A-blood	183 (146-276)	314 (121-535)[a]	165 (79-264)[b]	0.037
P1-blood	155 (59-169)[a]	63 (37-84)[b]	38 (20-91)[b]	0.015
P2-blood		29 (16-49)	19 (9-39)	0.40
CD4/CD8 ratio				
A-blood	1.14 (1.01-1.43)[a]	1.50 (0.97-1.96)[a]	0.40 (0.32-0.68)[b]	0.0001
P1-blood	1.34 (1.30-1.87)[a]	1.45 (1.14-2.17)[a]	0.43 (0.34-0.71)[b]	0.0001
P2-blood		1.61 (1.16-2.27)	0.46 (0.33-0.70)	0.0002

Variables presented as median (interquartile range [IQR]), different lowercase superscripts denote significantly different groups (where three groups were compared), same letters indicate no significant difference

more resulted in a significantly higher level of septic and surgical postoperative complications in the total study population. In the subgroup analysis of the HIV-seropositive and HIV-seronegative patients, only the HIV-negative patients showed significantly more septic complications after a drop of the NK cells of 50% or more ($P = 0.029$).

Discussion

In this study we determined how the main circulating immune cells change in the acute phase following penetrating abdominal trauma and whether there is a difference in the immune cell profile between HIV-seropositive and -negative patients. It has to be taken into account that posttraumatic lymphocytic changes are part of a physiological response of the immune system in order to control the sustained impact.

Special focus was put on the role of the NK cells. Furthermore, it was investigated whether the extent of these changes affected the rate of postoperative septic and surgical complications. Regardless of HIV-status, CD4+ T cells, CD8+ T cells and NK cells had the most severe decrease between admission and relook operation while the B cell numbers were only affected marginally. CD4+ T cell changes were more extensive in HIV-negative- than in HIV-seropositive patients. Of all examined immune cells, only a more severe decrease in NK cells within the first 48h was associated with more postoperative septic complications.

In addition to the trauma burden, HIV-seropositive patients are further jeopardized by their preexistingly compromised immune system. This was reflected in a significantly lower CD4+ T cell count in their admission blood compared to the HIV-negative patients.

The CD4+ T cell count in the seropositive group was only slightly higher for the patients on ARVs compared to the treatment-naïve patients. The numbers in a range between 300 and 400 cells/μL are comparable to numbers derived from an evaluation of 1310 HIV- patients of rural KwaZulu-Natal in South Africa [16]. Notably, the study patients of our research project were almost exclusively male, who on average have a significantly lower CD4-counts compared to females, as analyzed in the above-mentioned study. Moreover, there is evidence that a high percentage of South African patients on ARVs have an inadequate rise of their CD4 count in their peripheral blood despite full viral suppression. The main reasons identified for the incomplete reconstitution in the peripheral blood are advanced age and low CD4 cell count nadir on treatment initiation [17]. Since our patients were not of advanced

Table 3 – Lymphocyte decrease between A and P2-blood according to HIV-serostatus.

Quantity (cells/μL)	HIV-seronegative patients ($n = 16$)	HIV-seropositive patients ($n = 10$)
CD3+		
A-blood	1370 (925-2152)	1193 (888-2309)
P2-blood	317 (239-453)	331 (274-594)
P-value	0.0004	0.0051
CD4+		
A-blood	747 (444-1130)	320 (240-407)
P2-blood	188 (127-279)	118 (80-183)
P-value	0.0005	0.0069
CD8+		
A-blood	469 (315-852)	824 (486-1823)
P2-blood	110 (74-168)	198 (149-450)
P-value	0.0004	0.0051
B-cell		
A-blood	189 (114-317)	104 (67-149)
P2-blood	154 (118-280)	91 (43-125)
P-value	0.11	0.33
NK-cells		
A-blood	412 (213-556)	133 (83-258)
P2-blood	38 (19-52)	19 (9-39)
P-value	0.0004	0.0051
CD4/CD8 ratio		
A-blood	1.23 (0.77-1.85)	0.29 (0.18-0.58)
P2-blood	1.76 (1.33-2.43)	0.46 (0.33-0.70)
P-value	0.0035	0.15

Variables presented as median (interquartile range [IQR])

age, the main reason for the low admission CD4+ T lymphocyte count in the patients on ARVs may have been a delayed start of the antiretroviral treatment.

In the admission blood, the NK-cell numbers of the HIV-negative patients were higher than those of the control- and the HIV-seropositive patients, reaching a significant level between the HIV-negative and HIV-seropositive groups. NK-cells are predominantly activated by interferon and IL-12. They are also strongly affected by catecholamines, to a much higher extent than other lymphocyte subsets. There is evidence that trauma initially leads to a catecholamine-induced mobilization of NK cells from the spleen, bone marrow, lung, lymphatic tissue and marginal blood pool into the circulating blood, which might explain this early increase among the HIV-seronegative patients [18,19]. Lower initial NK-cell numbers in the HIV-seropositive patients might be due to HIV-induced quantitative alterations, especially of the CD56+ NK cell subset. Together with a disturbed cytokine pattern, this might lead to a reduced capacity to mobilize these cells in the initial trauma phase in response to the increased catecholamines [20,21].

In the P1- blood, a significant decrease of more than half of the T-lymphocytes and NK-cells was documented in the HIV-negative patients, while the B-lymphocytes declined only marginally. Notably the drop of T-lymphocytes was significantly higher in the HIV-negative compared to the HIV-seropositive patients. In the P2-blood that was taken 4 days after injury we demonstrated an almost complete depletion of NK-cells irrespective of the HIV-status. This early severe decrease after trauma is not supported by previous studies that describe only a reduced NK-cell *activity* [6] and not this substantial drop in absolute numbers. In homeostasis NK-cells monitor lymphoid and non-lymphoid organs; a possible explanation for the severe decrease might be a sequestration of these cells to the site of injury and inflammation [22]. It has also been described that activated NK cells become more adherent to the vascular epithelium which may also have contributed to the massive drop of circulating and detectable cells in the blood stream [23].

In determining which perioperative factors have the strongest influence on quantitative changes in the immune cells, we report that only the duration of inotropes had a significant impact for the majority of the analyzed immune cells. Patients who required ongoing inotropic support for more than 48 hours had a significantly lower decrease of T- and B-lymphocytes. The less pronounced lymphocyte fall in the acute phase might be explained by adrenaline-induced mobilization of lymphocytes from a marginated pool into the peripheral blood. This influx is thought to derive mainly from the spleen, bone marrow, and walls of high-endothelial venules [24].

Injury severity did not result in significant changes in the immune cell quantity in our study, hence results from previ-

Table 4 – Influence of lymphocyte quantity on admission on septic complications.

Total ($n = 62$)	No septic complications ($n = 20$)	Septic complications ($n = 42$)	P-value
CD3+ T cells	1815 (787-2301)	1280 (727-2152)	0.22
CD4+ T cells	816 (369-1328)	450 (256-772)	0.0094
CD8+ T cells	721 (316-907)	523 (285-1092)	0.96
CD4/CD8 ratio	1.30 (0.97-2.02)	0.73 (0.37-1.37)	0.0044
B-cells	226 (142-272)	135 (82-252)	0.049
NK-cells	261 (108-579)	196 (106-422)	0.46
HIV-seronegative ($n = 38$)	($n = 18$)	($n = 20$)	
CD3+ T cells	1904 (847-2301)	1286 (747-2182)	0.32
CD4+ T cells	825 (588-1328)	715 (365-1066)	0.22
CD8+ T cells	721 (328-898)	451 (281-974)	0.60
CD4/CD8 ratio	1.55 (1.17-2.02)	1.37 (0.77-1.90)	0.35
B-cells	238 (150-272)	189 (127-386)	0.55
NK-cells	261 (108-579)	358 (143-497)	0.57
HIV-seropositive ($n = 24$)	($n = 2$)	($n = 22$)	
CD3+ T cells	1039 (584-1494)	1187 (707-1880)	0.75
CD4+ T cells	289 (238-340)	338 (196-482)	0.75
CD8+ T cells	707 (316-1098)	571 (326-1314)	0.83
CD4/CD8 ratio	0.53 (0.31-0.75)	0.40 (0.32-0.65)	0.88
B-cells	157 (88-226)	120 (76-157)	0.68
NK-cells	238 (206-269)	140 (76-258)	0.28

Variables presented as median (interquartile range [IQR])

ous research that showed a significant influence of this variable could not be confirmed [5]. The investigation which immune cells have a prognostic impact on the outcome revealed for the admission blood the importance of the CD4+ T lymphocyte count: patients with no postoperative complications had significantly higher CD4+ T cell counts on admission than those with complications.

During the postoperative course a more extensive decrease of NK-cells in the study group was significantly associated with more septic and surgical postoperative complications. The main function historically attributed to NK cells has been protection against virus-infected- and tumor cells. NK cells may either stimulate and promote differentiation of T cells or may inhibit T cell activation and even induce apoptosis of these immune cells. This effect takes place via direct cell to cell interaction or is cytokine-mediated [25]. The importance of NK cells for fungal immunity as well as their antibacterial properties has become more evident over the last years [26,27], but is not without controversy.

Ebbo *et al.* demonstrated that patients with low NK cell counts are at higher risk for invasive bacterial infections [28]. However, there is evidence from a murine model that showed improved outcomes after induced depletion of the NK cells: Barkhausen *et al.* demonstrated this impressively by having a decreased mortality after induced NK cell depletion in a murine trauma study. An improved outcome after depletion was associated with a lower IL-6 level, which is a central cytokine in the inflammatory response, and less lymphocyte apoptosis [29].

Moreover it has been documented that NK cell depletion results in higher numbers of CD8+ T cells which correlates with an improved function of antigen presenting cells and therefore a quicker clearance of an evolving infection [30]. However, our data demonstrated the opposite: the NK cell decrease correlated significantly, positively with the CD8+ T cell decrease.

Current HIV research has provided evidence that NK-cells are involved in HI-virus control and suppression and play as important a role as CD8+ T cells. Via crosstalk with dendritic and other effector cells and via secretion of certain chemokines, NK-cells are able to destroy HIV-infected target cells and suppress viral replication [31,32], hence the posttraumatic NK-cell decrease might affect the disease course of HIV-seropositive patients losing one more pillar of their armamentarium in the fight against HIV-infection.

Since we observed in the total study population that patients with a more severe decrease of NK cells had more septic complications, we are not able to confirm the results of the abovementioned murine model and doubt whether they can simply be translated into the human and trauma context. In fact, our findings encourage an approach of boosting the NK cell function with the aim of strengthening the immune system to control bacterial infections.

There are various options to enhance the NK cell function; either directly by targeting active ligands on the cell itself, such as the NKG2D-ligand, by the blockade of inhibitory receptors or indirectly via cytokine-driven activation. Immunotherapy with re-transfusion of externally expanded NK cell popu-

Table 5 – The extent of lymphocyte decrease on postoperative outcome.

% decrease in cellular subtype	Number of septic complications			Clavien-Dindo classification		
Total ($n = 58$)	0 or 1 ($n = 25$)	2 or more ($n = 33$)	*P*	0-2 ($n = 25$)	3-5 ($n = 33$)	*P*
CD3+ T cells			0.59			0.59
<50%	8 (38%)	13 (62%)		8 (38%)	13 (62%)	
≥50%	17 (46%)	20 (54%)		17 (46%)	20 (54%)	
CD4+ T cells			0.78			>0.99
<50%	10 (48%)	11 (52%)		9 (43%)	12 (57%)	
≥50%	15 (41%)	22 (59%)		16 (43%)	21 (57%)	
CD8+ T cells			0.79			0.79
<50%	9 (39%)	14 (61%)		9 (39%)	14 (61%)	
≥50%	16 (46%)	19 (54%)		16 (46%)	19 (54%)	
B-cells			>0.99			0.50
<50%	20 (43%)	27 (57%)		19 (40%)	28 (60%)	
≥50%	5 (45%)	6 (55%)		6 (55%)	5 (45%)	
NK-cells			0.042			0.006
<50%	8 (73%)	3 (27%)		9 (82%)	2 (18%)	
≥50%	17 (36%)	30 (64%)		16 (34%)	31 (66%)	
HIV-seronegative ($n = 34$)	0 or 1 ($n = 21$)	2 or more ($n = 13$)	*P*	0-2 ($n = 18$)	3-5 ($n = 16$)	*P*
CD3+ T cells			0.68			>0.99
<50%	5 (71%)	2 (29%)		4 (57%)	3 (43%)	
≥50%	16 (59%)	11 (41%)		14 (52%)	13 (48%)	
CD4+ T cells			0.70			0.72
<50%	7 (70%)	3 (30%)		6 (60%)	4 (40%)	
≥50%	14 (58%)	10 (42%)		12 (50%)	12 (50%)	
CD8+ T cells			0.44			0.69
<50%	6 (75%)	2 (25%)		5 (63%)	3 (37%)	
≥50%	15 (58%)	11 (42%)		13 (50%)	13 (50%)	
B-cells			0.68			0.42
<50%	16 (59%)	11 (41%)		13 (48%)	14 (52%)	
≥50%	5 (71%)	2 (29%)		5 (71%)	2 (29%)	
NK-cells			0.029			0.090
<50%	7 (100%)	0 (0%)		6 (86%)	1 (14%)	
≥50%	14 (52%)	13 (48%)		12 (44%)	15 (56%)	
HIV-seropositive ($n = 24$)	0 or 1 ($n = 4$)	2 or more ($n = 20$)	*P*	0-2 ($n = 7$)	3-5 ($n = 17$)	*P*
CD3+ T cells			0.62			>0.99
<50%	3 (21%)	11 (79%)		4 (29%)	10 (71%)	
≥50%	1 (10%)	9 (90%)		3 (30%)	7 (70%)	
CD4+ T cells			0.30			>0.99
<50%	3 (27%)	8 (73%)		3 (27%)	8 (73%)	
≥50%	1 (8%)	12 (92%)		4 (31%)	9 (69%)	
CD8+ T cells			>0.99			>0.99
<50%	3 (20%)	12 (80%)		4 (27%)	11 (73%)	
≥50%	1 (11%)	8 (89%)		3 (33%)	6 (67%)	
B-cells			>0.99			>0.99
<50%	4 (20%)	16 (80%)		6 (30%)	14 (70%)	
≥50%	0 (0%)	4 (100%)		1 (25%)	3 (75%)	

(*continued on next page*)

Table 5 – (*continued*)

% decrease in cellular subtype	Number of septic complications			Clavien-Dindo classification		
Total ($n = 58$)	0 or 1 ($n = 25$)	2 or more ($n = 33$)	*P*	0-2 ($n = 25$)	3-5 ($n = 33$)	*P*
NK-cells			0.54			0.059
<50%	1(25%)	3 (75%)		3 (75%)	1 (25%)	
≥50%	3 (15%)	17 (85%)		4 (20%)	16 (80%)	

Categorical variables expressed as absolute and relative frequencies.

lations or transfusion of cryopreserved NK cells is under clinical investigation and may be especially beneficial for immunocompromised patients [33,34]. The main problem of this therapeutic approach is a possible graft-versus-host-disease and the long-term storage of these cells [35,36].

This study was not without limitations. In the flow cytometric analysis all the lymphocytes that were CD3 negative and CD56 positive were counted and reported as NK cells; sub analysis and differentiation into $CD56^{bright}$ and $CD56^{dim}$ NK-cells were not conducted. Nonetheless, with the antibodies that were used, a correct reflection of the total quantity of circulating NK cells in the blood was achieved and the changes in the perioperative phase could be documented.

The patient number of this research project was only powered to determine of large size effects, in the subgroup analysis we might have been missed significant associations, not because they did not exist but due to the small numbers. This was in particular true for the HIV-seropositive patients who formed the smallest subgroup. Furthermore, the HIV-seropositive patients were not age-matched with the HIV-seronegative patients, the HIV-seropositive patients being significantly older than the HIV-seronegative patients, which needs to be considered in the interpretation of our data since the factor age itself results in a higher immune risk profile with lower circulating immune cells [37]. However clinical significance is only to be expected in higher age groups and hence should not have biased our results since our patients weren't of advanced age.

Conclusions

Our study shows that the impact of penetrating abdominal trauma leads to an almost complete depletion of NK cells in the blood. Patients with a more extensive NK cell decrease had a significantly higher rate of septic and surgical complications, suggesting the important role of these cells for antibacterial immunity. Strengthening the NK cell function or limiting their decrease in the postoperative course might be of therapeutic value in severe trauma patients and is worth exploring in future research.

Author Contributions

Deirdré Kruger: Drafting and revising the article, support in statistical analysis

Sugeshnee Pather: protocol development, conception of study and acquisition of data Frank Plani: designing the study and acquisition of data, constant advise and surveillance of the project, data evaluation

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REFERENCES

1. Wafaisade A, Lefering R, Bouillon B, et al. Epidemiology and risk factors of sepsis after multiple trauma: an analysis of 29,829 patients from the trauma registry of the German society for trauma surgery. *Crit Care Med*. 2011;39:621–628.
2. Heffernan DS, Monaghan SF, Thakkar RK, Machan JT, Cioffi WG, Ayala A. Failure to normalize lymphopenia following trauma is associated with increased mortality, independent of the leukocytosis pattern. *Crit Care*. 2012;16:R12.
3. Manson J, Cole E, De De'Ath HD, et al. Early changes within the lymphocyte population are associated with the development of multiple organ dysfunction syndrome in trauma patients. *Crit Care*. 2016;20:176.
4. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol*. 2008;9:503–510.
5. Walsh DS, Siritongtaworn P, Pattanapanyasat K, et al. Lymphocyte activation after non-thermal trauma. *Br J Surg*. 2000;87:223–230.
6. Joshi P, Hauser CJ, Jones Q, et al. Mechanism of suppression of natural killer cells activity in trauma patients. *Res Commun Mol Pathol Pharmacol*. 1998;101:241–248.

7. Blazar BA, Rodrick ML, O'Mahony JB, Wood JJ BP. Suppression of natural killer-cell function following thermal and traumatic injury. *J Clin Immunol*. 1986;6:26–36.
8. Scharton TM, Scott P. Natural killer cells are a source of interferon γ that drives differentiation of CD4+ T cell subsets and induces early resistance to leishmania major in mice. *J Exp Med*. 1993;178:567–577.
9. Bowley DM, Cherry R, Snyman T, et al. Seroprevalence of the human immunodeficiency virus in major trauma patients in Johannesburg. *South African Med J*. 2002;92:792–793.
10. Onyekwelu I, Yakkanti R, Protzer L, et al. Surgical wound classification and surgical site infections in the orthopaedic patient. *JAAOS Glob Res Rev*. 2017;1:e022.
11. Moore EE, Dunn EL, Moore JB, Thompson SJ. Penetrating abdominal trauma index. *J Trauma*. 1981;21:439–445.
12. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg*. 2004;240:205–213.
13. Glenncross DK, Janossy G, Coetzee L, et al. Large-scale affordable PanLeucogated CD4+ testing with proactive internal and external quality assessment: in support of the South African national comprehensive care, treatment and management programme for HIV and AIDS. *Cytometry Part B (Clinical Cytometry)*. 2008;74B(1):40–64 Suppl.
14. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39:175–191.
15. Mauser M, Kruger D, Pather S, Plani F. Compromised gut associated lymphoid tissue is a risk factor for postoperative septic complications in HIV-seropositive trauma patients. *World J Surg*. 2021;45:1006–1013.
16. Malaza A, Mossong J, Bärnighausen T, Viljoen J, Newell ML. Population-based CD4 counts in a rural area in South Africa with high HIV prevalence and high antiretroviral treatment coverage. *PLoS One*. 2013;8:e70126.
17. Julg B, Poole D, Ghebremichael M, et al. Factors predicting discordant virological and immunological responses to antiretroviral therapy in HIV-1 clade C infected zulu/xhosa in South Africa. *PLoS One*. 2012;7:e31161.
18. Schedlowski M, Hosch W, Oberbeck R, et al. Catecholamines modulate human NK cell circulation and function via spleen-independent beta 2-adrenergic mechanisms. *J Immunol*. 1996;156:93–99.
19. Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M. Catecholamine-induced leukocytosis : early observations, current research, and future directions. *Brain, Behav Immun*.. 1996;10:77–91.
20. Hu PF, Hultin LE, Hultin P, et al. Natural killer cell immunodeficiency in HIV disease is manifest by profoundly decreased numbers of CD16+CD56+cells and expansion of a population of CD16dimCD56- cells with low lytic activity. *J Acquir Immune Defic Syndr Hum Retrovirology*. 1995;10:331–340.
21. Søndergaard SR, Ullum H, Skinhøj P, Pedersen BK. Epinephrine-induced mobilization of natural killer (NK) cells and NK-like T cells in HIV-infected patients. *Cellular Immunol*. 1999;197:91–98.
22. Grégoire C, Chasson L, Luci C, et al. The trafficking of natural killer cells. *Immunol Rev*. 2007;220:169–182.
23. Allavena P, Paganin C, Martin-Padura I, et al. Molecules and structures involved in the adhesion of natural killer cells to vascular endothelium. *J Exp Med*. 1991;173:439–448.
24. Crary B, Hauser SL, Borysenko M, et al. Epinephrine-induced changes in the distribution of lymphocyte subsets in peripheral blood of humans. *J Immunol*. 1983;131:1178–1181.
25. Crouse J, Xu HC, Lang PA, Oxenius A. NK cells regulating T cell responses: mechanisms and outcome. *Trends Immunol*. 2015;36:49–58.
26. Schmidt S, Tramsen L, Lehrnbecher T. Natural killer cells in antifungal immunity. *Front Immunol*. 2017;8:1623.
27. Schmidt S, Ullrich E, Bochennek K, Zimmermann SY, Lehrnbecher T. Role of natural killer cells in antibacterial immunity. *Expert Rev Hematol*. 2016;9:1119–1127.
28. Ebbo M, Gérard L, Carpentier S, et al. Low circulating natural killer cell counts are associated with severe disease in patients with common variable immunodeficiency. *EBioMedicine*. 2016;6:222–230.
29. Barkhausen T, Frerker C, Pütz C, et al. Depletion of NK cells in a murine polytrauma model is associated with improved outcome and a modulation of the inflammatory response. *Shock*. 2008;30:401–410.
30. Cook KD JK. The depletion of natural killer cells prevents T cell exhaustion to efficiently control disseminating virus infection. *J Immunol*. 2013;190:641–649.
31. Tasca S, Tambussi G, Nozza S, et al. Escape of monocyte-derived dendritic cells of HIV-1 infected individuals from natural killer cell-mediated lysis. *AIDS*. 2003;17:2291–2298.
32. Kottilil S, Chun T, Moir S, et al. Innate immunity in human immunodeficiency virus infection: effect of viremia on natural killer cell function. *J Infect Dis*. 2003;187:1038–1045.
33. Souza-Fonseca-Guimaraes F, Adib-Conquy M, Cavaillon JM. Natural killer (NK) cells in antibacterial innate immunity: angels or devils? *Mol Med*. 2012;18:270–285.
34. Freund-Brown J, Chirino L, Kambayashi T. Strategies to enhance NK cell function for the treatment of tumors and infections. *Crit Rev Immunol*. 2018;38:105–130.
35. Lehrnbecher T, Kalkum M, Champer J, et al. Immunotherapy in invasive fungal infection-focus on invasive aspergillosis. *Curr Pharm Des*. 2013;19:3689–3712.
36. S Szmania S, Lapteva N, Garg T, et al. Ex vivo-expanded natural killer cells demonstrate robust proliferation in vivo in high-risk relapsed multiple myeloma patients. *J Immunother*. 2015;38:24–36.
37. Wikby A, Månsson IA, Johansson B, Strindhall J, Nilsson SE. The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20-100 years of age. *Biogerontology*. 2008;9:299–308.