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Dynamics of leukocyte receptors after severe burns: an exploratory study

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Abstract:

Background: Patients with burns are susceptible to organ failure, and there is indirect evidence that leukocytes may contribute to this process. They may change the expression of cell-surface receptors after certain stimuli- for example, the burn. We therefore aimed to assess the changes induced by the burn in the expression of leukocyte cell-surface receptors CD11b, CD14, CD16, and CD62L on the surface of PMNs and monocytes. We also wanted to examine the dynamics of this activation during the first week after the burn, and to relate it to the size of the injury.

Methods: Ten patients with burns of > 15% (TBSA) were included in the study. Blood samples were collected on arrival and every consecutive morning during the first week. Healthy volunteers acted as controls.

Results: PMN CD11b expression was increased. The extent of PMN CD11b expression correlated negatively to the size of the full thickness burn.

Monocyte CD14 expression increased initially but there was no relation to the size of the burn. PMN CD16 expression decreased initially during the first days and the decrease was related to burn size.

CD 62L did not vary depending on the burn in either PMN or monocytes during the first week after the burn.

Conclusion: This study showed that specific receptors on the surface of leukocytes (PMN CD 11b, monocyte CD 14 and PMN CD 16) are affected by the burn. Expression of PMN CD11b and CD16 are related to burn size. Burn-induced effects on the expression of PMN receptors, such as PMN CD11b and CD 16, may contribute to burn-induced infection susceptibility.

Abbreviations:

Fc	Constant region on Ig
FTB%	Full thickness burn in % of body area
LPS	Lipopolysaccharide
MFI	Mean fluorescence intensity
MOF	Multiple organ failure
PMN	Polymorphonuclear leukocytes
TBSA%	Total burned surface area in % of body area
TLR	Toll-Like receptor

Introduction

Victims of burns are susceptible to multiple organ dysfunction syndrome (MODS) and multiple organ failure (MOF). MODS/MOF is the most common factor today that leads to morbidity and mortality after burns [1]. This syndrome is well-characterised for burned patients, and for patients who need intensive care for other critical illnesses. However, the underlying pathophysiology of MODS/MOF is still not clear and widely debated. Organ failure after burns may appear early, as a recent publication has shown [2], or late [3]. The early failure manifests itself mainly by increased vascular permeability in the lungs, leading to acute respiratory distress syndrome, and occurs in a span of 1-48 hours after the burn. In this phase of the treatment patients are most often not septic and the organ failure seems related to the burn itself. Later in the course of treatment, patients may develop sepsis and organ failure related to this.

Earlier work indicated that leukocytes, PMNs in particular, may be involved in the development of organ failure after injuries other than burns, such as blunt trauma [4-5]. Recent work from our group has shown that the degree of respiratory failure after burns correlates with dynamic changes in the white blood count [2]. There are therefore many indirect indications that the effects of leukocytes may lead to organ dysfunction, but the exact mechanisms remain unclear.

There is a large increase in WBC early after the burn, which is followed by a reduction to subnormal levels that is sustained for more than a week [6-7]. Animal models of burns have indicated that there are many extravasated PMNs in lungs, kidneys, liver, brain, and skin [8-9]. Hansbrough et al. investigated the kinetics of this invasion of PMNs in lungs and found the maximal number of PMNs at 8 hours after the injury [9]. In the later phase, when WBC are below normal, others have shown evidence of immune suppression that manifest itself in

many different ways [6, 10-11], which seems to be at least in part related to the inability of PMNs to upregulate CD11b and maintain CD16 expression [12].

Leukocytes may be activated by different stimuli, and then change their expression of cell-surface receptors.

We therefore hypothesised that there is a standardized change in the expression of leukocyte receptors expression after severe burns. To examine this we aimed to analyse 4 leukocyte cell surface receptors on PMNs and monocytes in 10 severely burned patients.

CD62L mediates rolling and CD11b mediates firm adhesion of PMNs to the endothelial cells [13], hence both are critically involved in the recruitment of PMNs to extravascular tissue.

CD 62L is, to our knowledge not characterised after burns and CD11b has been shown to increase after burns [14-16]. Since PMNs are found to infiltrate tissues after burns it is reasonable to suspect alterations in these receptors. CD 16 is an Fc-receptor found on PMNs and monocytes [17] that has been shown to decrease after burns earlier [14-15, 18]. The alteration of this receptor is thought to mediate the decreased ability of phagocytosis seen in PMNs after burns. CD14 is expressed predominantly on monocytes and is a co-receptor of TLR-4 which mediates lipopolysaccharide binding to monocytes [19]. With this binding it is thought to mediate some of the symptoms seen in sepsis. Since it has not been investigated in burns before, it was included in this investigation. Blood samples for these analyses were taken daily for one week after the injury.

Our aim was to identify which, if any, receptors that responded on either PMNs or monocytes, and which of them seemed to be related to the size of the burn and to further characterise this change.

After a major burn patients may have infectious complications including sepsis. This is especially true later in the course of burn care. To examine if our patients had sepsis we also analysed PCT (procalcitonin) which is a sensitive marker of bacteraemia [20-21].

Material and methods:

Ten consecutive patients were prospectively included. The study was done at Linköping University Hospital, which is one of two national burn units in Sweden. The study was approved by the local ethical committee and oral consent was sought from patients or their next of kin. Inclusion criterion was TBSA > 15% as calculated using a Lund Browder chart. Most patients come from a long way away from the hospital and their trauma may then be some hours old, and for separate cases as much as 24 hours before admission. It was therefore decided that a first blood sample would be drawn when the patient was admitted. The next six samples were drawn from the patients arterial catheter on consecutive mornings for six days. The time that elapsed from first to second sample varied from 16-24 hours.

Burn care

All patients were cared for by a protocol as previously presented [22]. They were resuscitated according to the Parkland formula (4 ml/kg/TBSA%). Early excision and grafting was started within 24-48 hours. Autologous skin transplants were performed when donor skin was available. Cadaver skin or pig skin was applied when there was a shortage of autologous skin. Enteral nutrition was instituted on admission. Regular dressings containing a combination of silver and Sulfadiazine (Flamazine®) were used and dressings were changed every second day.

Preparation of samples: A panel of 4 monoclonal antibodies conjugated with fluorescein-isothiocyanate (FITC) or phycoerythrin (PE) were used in combination to characterise the white blood cells in the daily blood sample. Autofluorescent control and isotype control, protein concentration, and S/P-ratio matching the antibodies were used. Anti (α) CD14-PE, α CD62L-FITC/CD11b-PE, and α CD16PE from Immunotech (Coulter Corp. Marseilles France) were used.

Blood 100 μ l in EDTA was incubated with 10 μ l of the titrated monoclonal antibody combinations at room temperature for 10 minutes. Optilyse B 100 μ l (Coulter Corp. Marseilles France) was added, the tube vortexed and left standing for another 10 minutes. Water 1000 μ l was then added and 10 minutes later the sample was analyzed using a FACSCalibur flow cytometer (BD, San Jose, California, USA).

Flow cytometry: Colour compensation was checked with the individual blood, stained with α CD8-FITC/CD4-PE (Coulter Corp. Marseilles France). Samples taken from blood donors in the Department for Transfusion Medicine were run in the same panel together with those from each patient and served as controls.

Variation in the cell numbers in duplicate runs were within 4% and variations in the MFI were less than 15%. The instrument itself, checked daily with Standard Brite (Coulter Corp. Marseilles France), did not vary from day to day more than 1%.

The concentration of procalcitonin was measured using an immunoluminometric assay kit from BRAHMS Aktiengesellschaft.

Data analysis and statistics

We tested the time variations of the 4 different receptor expressions using Friedman's ANOVA and Kendall's coefficient of concordance.

For the receptors that showed significant changes: PMN CD11b, monocyte CD14, and PMN CD16, we investigated the correlation between the receptor expression and size of injury.

A multiple regression model was used to check for a correlation between any of the receptor expressions of dependent variables PMN CD11b, monocyte CD14 and PMN CD16 with the independent variables TBSA% and FTB %. Since there is a dependency of time and a possible interpatient variability in the measured variables, the day and patient number was also included as independent variables.

Procedure used to check for cells that did not express a receptor:

For each specific receptor analysis, we analysed one irrelevant negative control-antibody. We then calculated a cut-off value. This cut-off value indicates the fluorescence value at which 98 % of the cells stained as negative control have lower fluorescence. With the aid of this cut-off value, we calculated the percentage of positive cells for each receptor and sample.

To help our data analysis we used the statistical package Statistica ver. 9.0 (Tulsa, USA).

Probabilities of less than 0.05 were accepted as significant.

Results

Demographic data and severity of burns are presented in Table I. Mean (SD) TBSA% of the 10 patients was 35,2 (22,3). Median TBSA% were 28 and median FTB% 15,5. All were flame injuries. One patient died: an 88-year-old woman who died 11 days after her 24 % TBSA burn.

The leukocyte count followed the common pattern after burns with a sharp initial rise followed by a decline to subnormal levels that were sustained during the study (data not shown).

PCT was generally below 7 ng/ml except in patient no. 4, who showed signs of a systemic infection days 6 and 7 (fig 1).

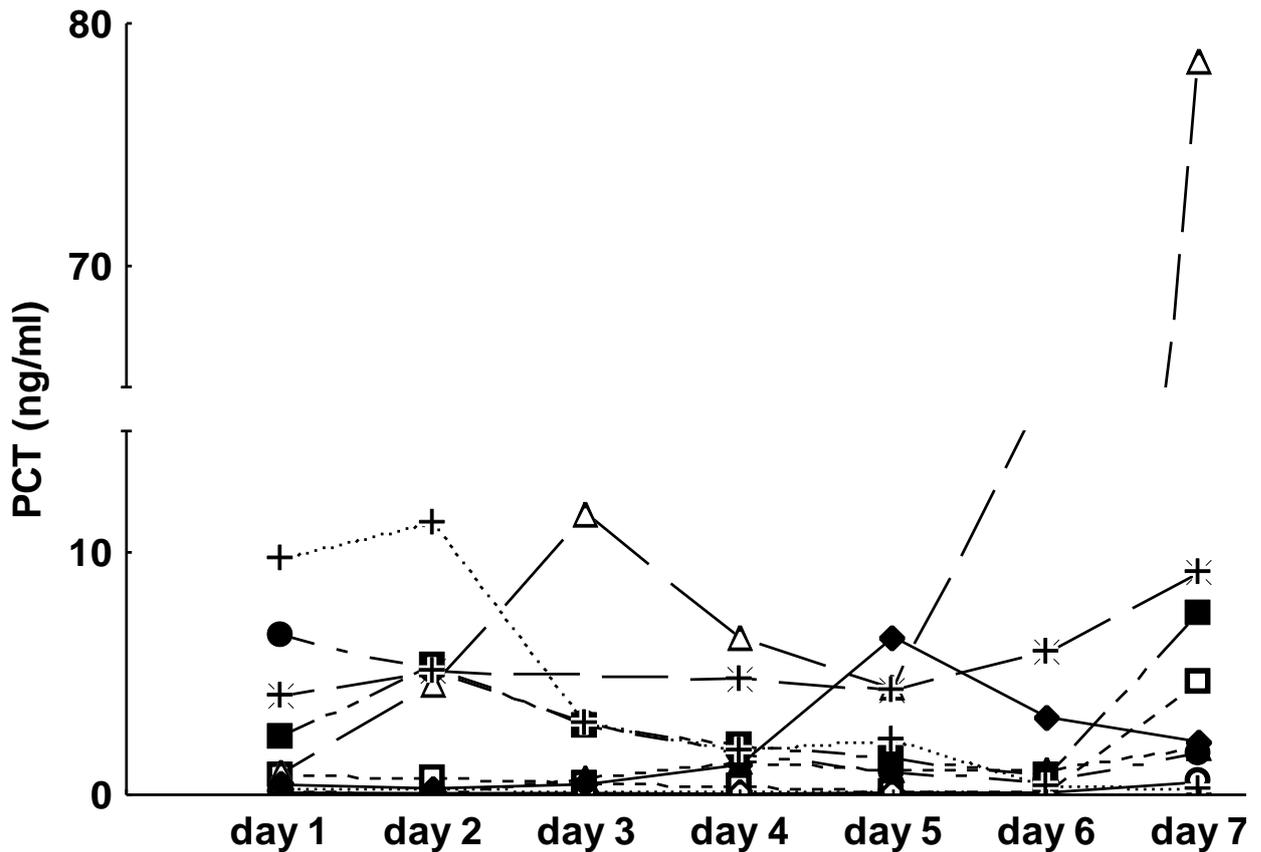


Figure 1: Variation of plasma procalcitonin (PCT) in 10 patients the first 7 days after a burn

Specific receptors

PMN CD11b

This receptor was expressed in control subjects on both PMNs and monocytes. The percentage of positive cells was above 95% in most cases and in all cases above 80% (data not shown).

There was increased expression in PMN CD11b after burns compared with control subjects, (fig 2). The increase was sustained through the first part of the study ($p = 0.03$). On days 6 to 7 expression decreased and was close to that of controls. PMN CD11b showed a significant correlation with FTB% ($\text{beta} = -0.578$ $p = 0.039$) but not with TBSA% ($\text{beta} = 0.497$ $p = 0.074$). There was no trauma-induced change in CD11b in monocytes ($p = 0.16$) (data not shown).

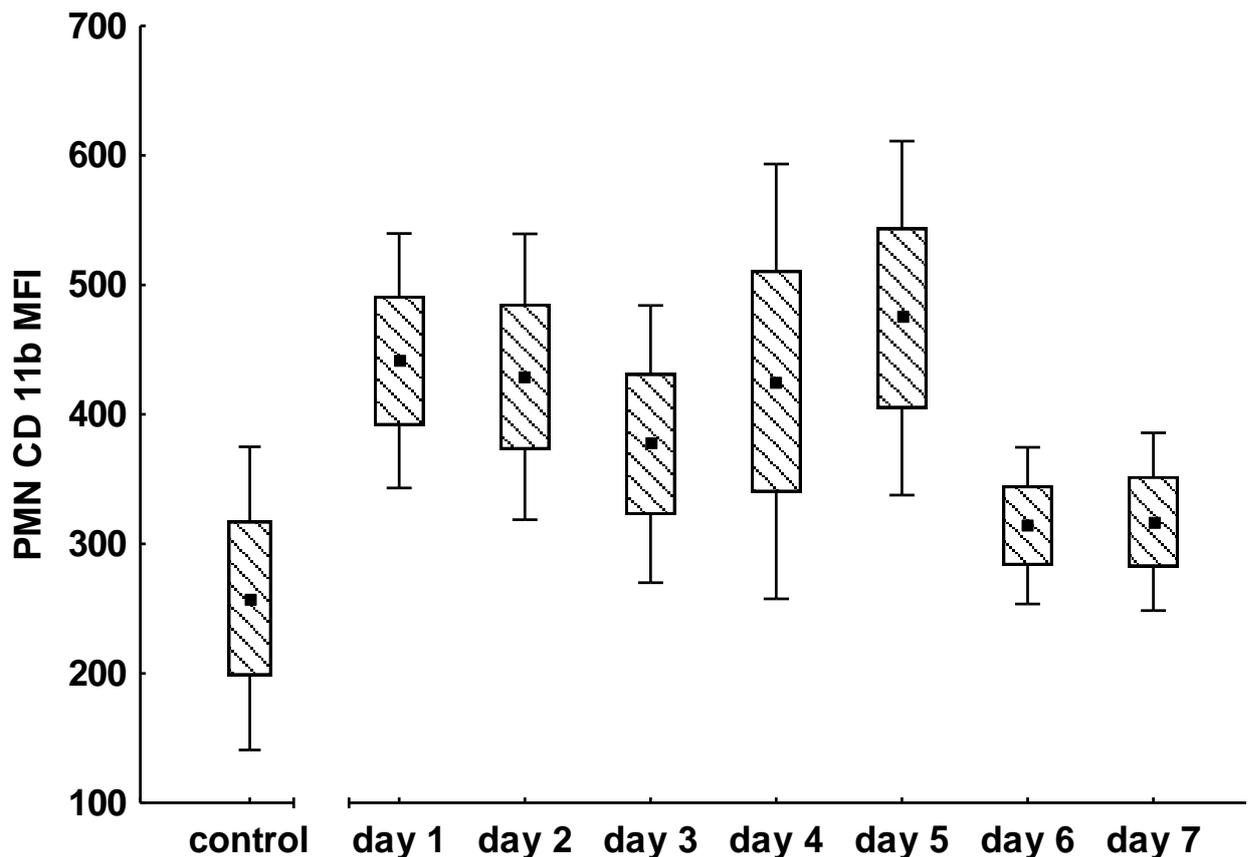


Figure 2: Variation of PMN CD11b the first 7 days after a burn. There was a significant relation to time, tested with Friedman ANOVA and Kendalls coefficient of concordance ($p = 0.033$). Square and bracket indicate standard deviation and 95% confidence interval.

CD14

Monocyte CD14 showed more than 80% of cells positive throughout our study except case 7, on day 7 in whom 70% of the cells were positive and case 4, day 3 in whom 78% of the cells

were positive. The monocyte count was so low in case 5 on day 5 to 7 that no data could be retrieved for CD14.

Monocyte CD14 expression changed over time ($p = 0.0006$) and remained high compared with controls during our study period, (fig 3).

There was no correlation between monocyte CD 14 and TBSA % ($\beta=0.132$ $p=0.611$) or FTB% ($\beta=-0.414$ $p=0.229$).

PMNs varied in the percentage of cells positive for CD14 (range 3%-86%) and the variation of individual MFI were also large. These data were not analysed further.

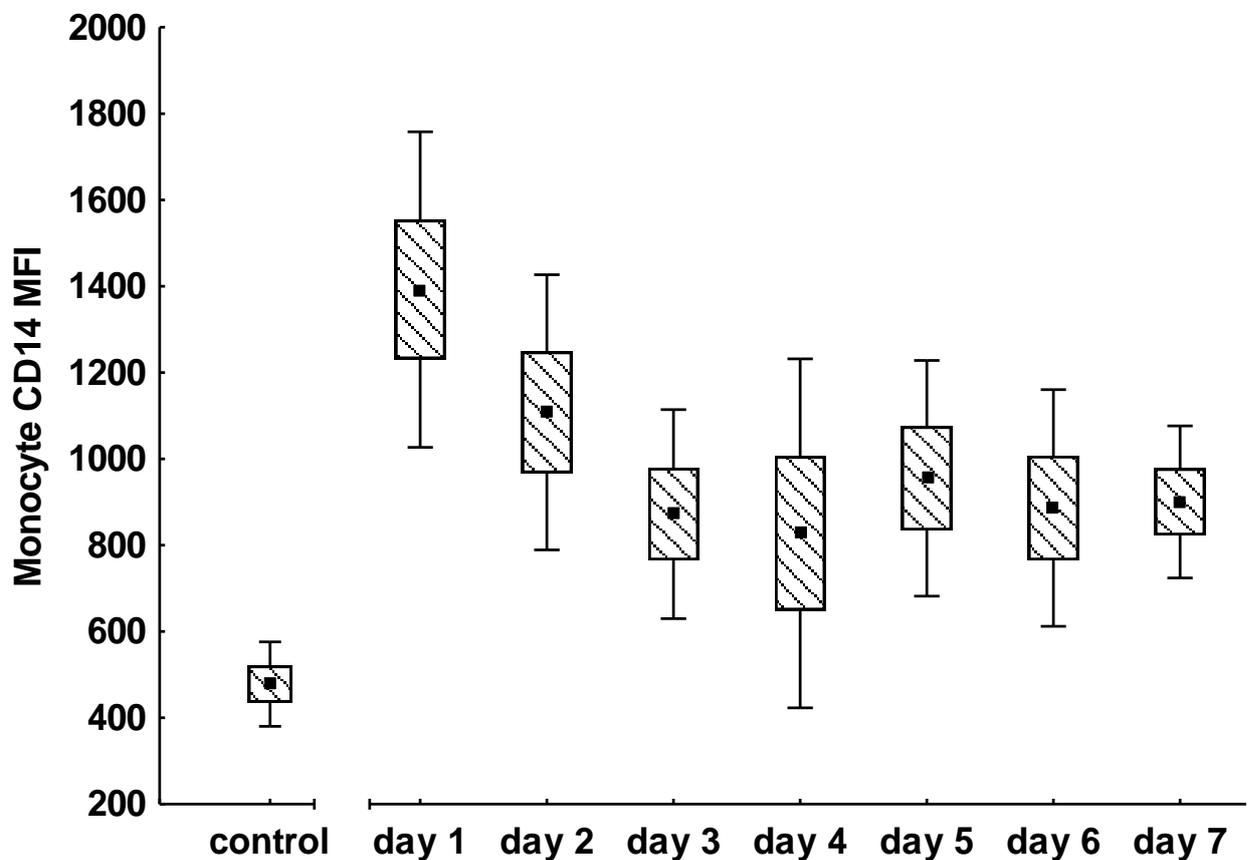


Figure 3: Variation of monocyte CD14 the first 7 days after a burn. There was a significant relation to time, tested with Friedman ANOVA and Kendalls coefficient of concordance ($p=0.000$). Square and bracket indicate standard deviation and 95% confidence interval.

CD16

PMN CD16 had > 95% of cells positive in most cases and > 80% of cells were positive in all cases.

PMN CD16 decreased consistently and this was sustained throughout the study period (p = 0.0001) (fig 4).

There was a significant relation between PMN CD16 and TBSA% (beta=0.564 p=0.032) and a negative a relation to FTB% (beta= -0.813 p=0.002)

Monocytes varied more in the percentage of cells positive for CD16 (range 6%-80%) and the variation in individual MFI were also large. These data were not analysed further.

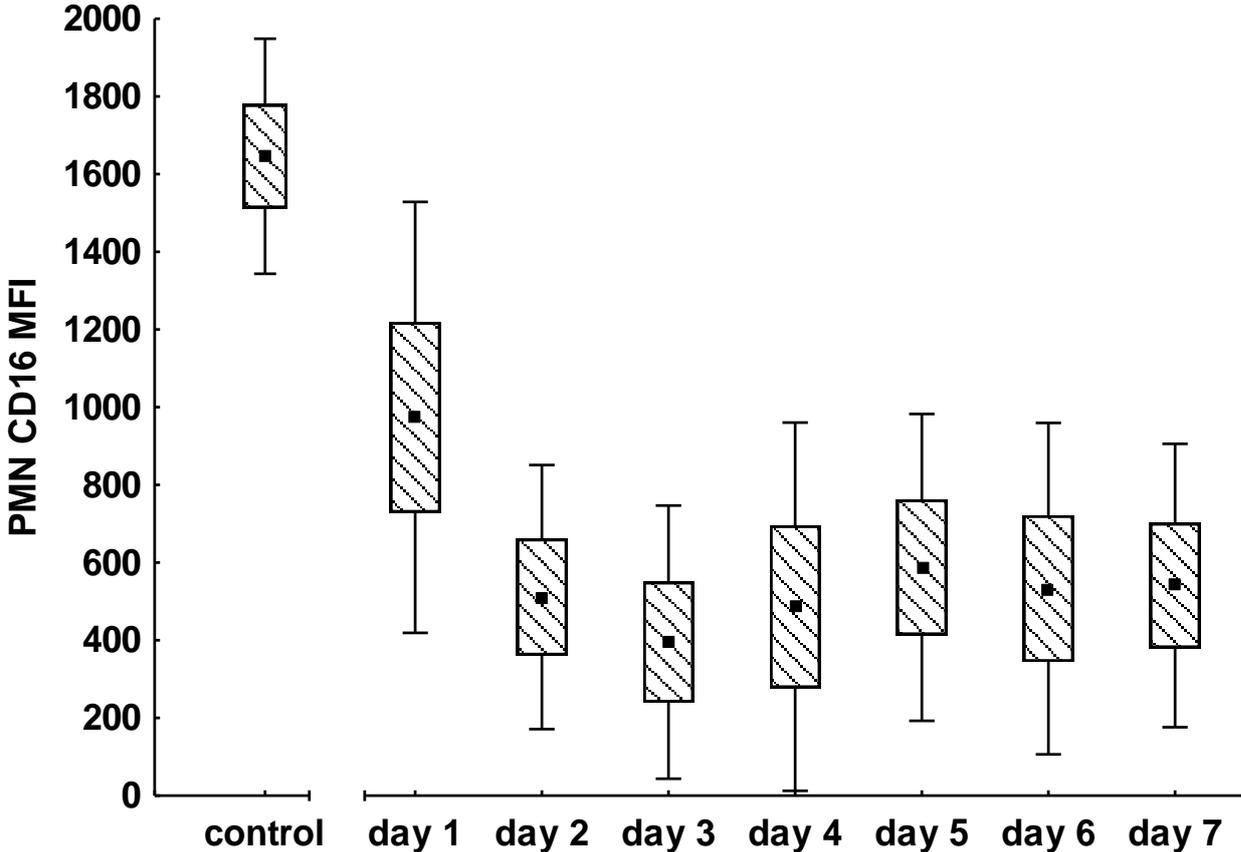


Figure 4: Variation of PMN CD16 the first 7 days after a burn. There was a significant relation to time, tested with Friedman ANOVA and Kendalls coefficient of concordance (p=0.000). Square and bracket indicate standard deviation and 95% confidence interval.

CD62L

There were no significant time-related variations in PMN ($p=0.473$) or monocyte ($p=0.06$) CD62L. Analyses of PMN and monocyte CD62L, however, were complicated by the fact that both showed large variations in the percentage of positive cells (range 1%-96 % and 10%-96 % respectively). These data were not analysed further.

Discussion:

In view of the dynamic changes seen in white blood cell counts after a burn it is interesting to examine expression of PMN and monocyte receptors. The findings underline the important impact that a burn injury places on these cells, and particularly the receptors that are claimed to be central to the function of them. Interestingly the expression of receptors seemed to follow distinct, burn related patterns. As described earlier [14-16], expression of PMN CD 11b increased after the burn as opposed to CD16, which decreased considerably. The dose response relation to burn size that was recorded suggests causality by the burn injury. The monocyte CD 14 expression showed a different pattern, as levels remained higher than in controls, but declined after the burn, and no dose response relation were detected.

CD11b and CD16 are stored in the walls of PMN granules and are up-regulated on PMNs when these granules fuse with the cell membrane. CD11b is found in secondary granules, tertiary granules, and secretory vesicles while CD16 is found exclusively in secretory vesicles [23-25]. The different subsets of PMN granules are formed during different phases of maturation of PMNs, secretory vesicles being the last to form during the last phase of the normally 2 week maturation of PMNs in the bone marrow before release into the blood.

In the case of severe burns, there is an early massive release of mature PMNs and the bone-marrow strives to keep up the amount of them that are circulating. There is also evidence of a depression of the bone marrow after burns in mice [7, 26], indicating that not only does the bone-marrow release all the mature PMNs available after burns, but it also has to produce new PMNs under non-optimal circumstances. An increased amount of circulating immature PMNs (bandforms), which probably lack secretory vesicles, may be the result, but to our knowledge this is shown only in patients after mechanical trauma [5] and not after burns. If this is true also for burns, it may explain our finding, confirmed by others, that CD16 is significantly decreased after burns. It may also explain that PMNs from burned patients cannot increase CD11b expression accurately [11], and this is in line with our finding of less increase of CD11b in larger full thickness burns.

Infectious complications may alter receptor expression on PMNs and monocytes. In our material this is not a likely explanation for the observed receptor alterations since the PCT-values strongly suggest that our patients in general do not have bacteraemia (fig 1). One of the patients had markedly increased PCT levels at day 6 and 7 indicating development of bacterial infection. It is, however, unlikely that this could affect the mean values of receptor expression.

Since PMN invasion of tissues is thought to mediate or contribute to early organ failure, the finding of increased PMN CD11b is interesting as this is the receptor used by PMNs to adhere to endothelium.

In a later phase of burn care, infection susceptibility and sepsis is common. Sepsis was shown by others to correlate with inability to increase PMN CD11b expression and inability to maintain PMN CD16 expression [12]. Therefore our finding of lesser PMN CD11b increase and a tendency for greater drop in PMN CD16 in larger full thickness burns is interesting, as

it may contribute to infection susceptibility and hence sepsis and tentatively to the development of late organ dysfunction.

In our analysis TBSA% and FTB% were regarded as independent variables. In the sense that FTB% can never exceed TBSA% they are not truly independent and this is a compromise.

However, as the study is based on a relatively small material, further analysis, i.e., comprising an interaction study was considered less relevant. As both measures were examined in parallel, we believe the risk of introducing an erroneous conclusion based on this procedure is small.

CD11b

Others have used similar methods to explore dynamics of CD11b soon after severe burns and their results are in line with ours, showing increased levels [14-16]. One investigation after mechanical trauma also show similar results [5].

The degree of CD11b expression is often used as a measurement of PMN activation in general. It is obvious that this expression alters the phenotype of the PMN but it is still not entirely clear how to interpret the effect of this increased expression on circulating PMNs.

CD11b has also been shown to regulate its affinity by inside-out signalling [27].

CD11b is the principal receptor used by PMNs to adhere to endothelial cells.

Earlier investigators have shown that a reduction in expression of this particular receptor on PMN in later stages of a burn precedes the onset of infection [12]. We therefore conclude that our finding of lesser increase in CD11b in larger burns (negative correlation to full thickness burn %) may contribute to the greater infection-susceptibility seen in larger full thickness burns.

Concerning this correlation between size of injury and expression of CD11b, Bjerkenes et al [15] showed a positive correlation between TBSA% and PMN CD11b but no correlation to FTB%. We have a trend towards a positive relation between PMN CD11b and TBSA% ($p=0.074$) and a significant negative relation with FTB%. The reason for this difference to our results is not clear. Their material differed from ours in that their mean TBSA% was larger and they did not use a multiple regression model but rather looked at every single day with a different test.

When discussing CD11b expression one must bear in mind that it is a highly dynamic event that may change in different stages of disease, and particularly in cases of infectious complications which is more common during later stages of burn care. These alterations are likely to be even more pronounced in patients in whom PMNs are increasingly margined or extravasated which is thought to be the case in those with burns.

CD14

Others have shown increased expression of CD14 in skin and other tissues after a burn [28-29] but, to our knowledge, the increased expression on blood monocytes that we found has not been reported before.

Studies have shown that mice deficient in TLR-4, of which CD14 is a co-receptor, survive an intravenous challenge with lipopolysaccharide (LPS) better than wild-type mice, but are increasingly sensitive to Gram-negative infection [30-31]. This is interesting, as it indicates that signalling through TLR-4/CD14 may be a crucial link that mediates some of the symptoms and response reactions seen in Gram-negative infection. Barber et al. have shown that this is the case for burn-induced cardiac dysfunction in a mouse model [32].

As our patients had an increased level of monocyte CD14 expression, our conclusion is that burned patients may respond more strongly to a Gram-negative bacteraemia. Sepsis is common in burned patients and contributes significantly to morbidity and mortality.

There are genetic polymorphisms that affect CD14 expression, and two groups have investigated the influence of the polymorphisms on morbidity and mortality after burns.

Barber et al found that CD14 159 C is associated with increased mortality [33] but Lin et al, could not confirm this [34].

The matter of CD14 expression is also complicated by the fact that CD14 may be shedded and enter the circulation as soluble CD14, of which Lin et al [34] found higher concentrations in those who died than in survivors after burns.

CD16

CD16 is also known as FcRIII. We found a marked reduction in PMN CD16 and this may contribute to the known susceptibility for infections that accompany larger burns. This is also in line with the findings of Vindenes et al. [18], who found decreased Ig-dependent phagocytosis after burns. The Ig-dependency is thought to be mediated by CD16 (and maybe other Fc-receptors).

PMN CD16 dynamics after severe burns has been investigated by others [15-16, 18] and their findings confirm ours with a rapid and large decline after a burn.

We had a positive relation of CD16 to TBSA% but a negative and stronger correlation to FTB%. We conclude that there seems to be physiological differences between the impact of a partial thickness and full thickness burn on PMN CD16 expression.

CD62L

There was large variation in the levels of CD62L expression on both PMNs and monocytes and no significant changes were detected over time or in relation to the size of the burn.

Physiological explanations for these variations may be either that expression is transiently alternating, shedding of the receptor which is described in SIRS earlier [35], or extravasation, where positive cells leave the circulation and are replaced by negative cells from the bone marrow.

Conclusion:

Here we show that PMN CD11b increases early after a burn and that there is a correlation between the increase and the size of the full thickness burn. PMN CD16 was shown to decrease early after a burn and there is a correlation to burn size. Monocyte CD14 was also found to vary in a significant and predictive way following the burn, although no significant correlation with the burn size was detected. CD62L did not vary in a predictive way after burns on either PMNs or monocytes.

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Table 1

Case No	Age at injury (years)	Percentage	
		Total burn surface area	Full-thickness burn
1 ¹	45	23	3
2	14	32	17
3	31	30	11,5
4	16	16	12,5
5	17	26	22
6	18	31	31
7	25	58	30
8 ²	88	24	14
9 ¹	22	22	1
10	20	90	70

¹ No ventilator treatment

² Died day 11

Legend: Demographic data and injury severity. All were flame injurys.