Published online: 2019-12-31

Original Article

Soluble CD163: A novel biomarker for the susceptibility to sepsis in severe burn injuries

Andrzej Piatkowski, Gerrit Grieb, Rittuparna Das[1], Ahmet Bozkurt, Dietmar Ulrich, Norbert Pallua

Department of Plastic Surgery and Hand Surgery, Burn Unit of the RWTH University Hospital in Aachen, Germany, [1]Department of Internal Medicine, Section of Infectious Disease, The Anlyan Center, 300 Cedar Street, Yale School of Medicine, New Haven, CT 06520, USA

Address for correspondence: Dr. Andrzej Piatkowski, Pauwelsstr. 30 Aachen Germany. E-mail: apiatkowski@ukaachen.de

ABSTRACT

Objective: Soluble CD163 (sCD163) has been previously shown to play a role in inflammatory and infectious diseases. This study, for the first time, investigates the characteristics and potential values of sCD163 in burn patients. A first look is taken on the changes of sCD163 levels in burn patients by comparing predefined subgroups at single time points. **Materials and Methods:** Serum samples of 18 patients with burn injuries were collected for biochemical analysis at the time of admission and in a chronological sequence of 12, 24, 48 and 120 h after the injury and were matched to clinical parameters. Statistical analysis was performed using the Mann-Whitney test, Wilcoxon signed rank and Pearson bivariate correlation. **Results:** Patients with sepsis showed a significant increase of sCD163 levels. sCD163 was correlated with leukocytes (P=0.035) over the time course of 120 h. Patients characterized by a burn size exceeding 25% of the total body surface area (TBSA) showed a significant increase of sCD163 between 12 and 48 h after burn injury (P=0.038). **Conclusions:** The first view on the characteristics of sCD163 in the serum of burn patients points out that sCD163 seems to be an early indicator for the susceptibility to sepsis. Furthermore, the changes in sCD163 serum levels within the first hours after burn trauma have great potential for early prediction of organ failure after burn injury.

KEY WORDS

Inflammation; immune response; organ failure; serum marker; sepsis

INTRODUCTION

A majority of thermal injuries result in a profound decrease of the immune capability, which predisposes burn patients to develop severe sepsis. This is due to liberation of various agents like endotoxins, hormones, cytokines and lymphokines with immunosuppressive properties into the circulation, which lead to a reduced function of monocyte and macrophage activity as well as a higher capacity of macrophages with suppressive functions. One of the points of interest in this context is the scavenger receptor CD163, which in its soluble form has been the centre of this study. Membranous CD163 is expressed predominantly on macrophages with an anti-inflammatory phenotype, so-called alternatively activated macrophages.[1] The membranous form functions as a scavenger receptor for haptoglobin-

Access this article online	
Quick Response Code:	Website: www.ijps.org
	DOI: 10.4103/0970-0358.81454

haemoglobin complexes.[2] Former studies have shown that there is a physiological expression of membranous CD163 on the surface of monocytes and macrophages.[3] Soluble CD163 (sCD163) is a new style serum marker originating exclusively from monocytes and macrophages.[4] It is a part of the membrane-bound CD163 receptor, which is shed into the extracellular space as a result of proteolytic cleavage near the cell membrane.[5-7] In vitro studies further have shown that in response to inflammatory stimuli an increased shedding of the extracellular part of CD163 (sCD163) occurs.[6,8,9] In the present study we investigated for the first time whether sCD163 serum levels showed a special course in burned patients with organ failure and/or sepsis during the first 120 h after injury. Furthermore, sCD163 was compared to the burned total body surface area (TBSA), abbreviated burn severity index (ABSI), and inflammatory markers, which have a value for the diagnosis of sepsis such as leukocytes, C-reactive protein (CRP) and procalcitonin (PCT). Moreover, we tried to compare plasma levels of sCD163 to the severity of organ dysfunction as estimated by the Severity Organ Failure Assessment *(*SOFA) score.

MATERIAL AND METHODS

Study design and patients

All patients who suffered from thermal injury >15% total body surface area (TBSA) were included in this study. Exclusion criteria were chemotherapy in the past, operative procedures during the past 2 weeks, a transfer to the burn centre longer than 2 h and individuals below the age of 18. Informed consent was obtained from every patient or their close relatives. Directly on admission, all patients were subjected to an initial debridement under general anaesthesia and the burned areas were photo-documented. Bronchoscopy was conducted initially on every patient to evaluate if an inhalation injury was present. Resuscitation was performed according to the Parkland formula using lactated Ringer´s solution over the first 24 h. The first blood samples were collected immediately after admission before the initial debridement by venous puncture. In the following days, blood samples were collected 12, 48 and 120 h post-admission. Further on all patients received one or up to two tangential excisions of the burned eschar. Epifascial excision was performed once on one patient. Temporary wound coverage was achieved by glycerol preserved donor skin (Euroskin) and wound dressing changes were performed daily. No steroids were given to the patients during the study period.

We investigated prospectively collected serum samples of 18 patients (6 females, 12 males), mean age 44.3 years with a mean burn size (total of second and third degree burns) of 47 % TBSA, admitted to our burn ICU. Patient's characteristics are shown in Table 1.

Table 1: Patient's characteristics

Characteristic	*Mean (range)*
Age (years)	44.3 (31-80)
Admission ABSI	9.4 (4-15)
TBSA (%)	47 (15-90)
Mortality (%)	22.2 (4/18)
Sepsis (%)	27.8 (5/18)

Each patient was examined for signs and symptoms of infection and microbiological analyses were performed at the time of admission and daily until they were discharged from the ICU or succumbed. Every day we classified all patients in one of the following three categories: non-systemic inflammatory condition, systemic inflammatory response syndrome (SIRS) or sepsis. Classification was performed according to the ACCP/SCCM criteria.[10] All patients were evaluated on abbreviated burn severity index (ABSI), total burn surface area (TBSA) and Sepsis-related organ failure assessment (SOFA) score on admission. Only the SOFA score was also evaluated after 12 h and daily until discharge from ICU. According to previous studies and based on the fact that extensive burn injuries with TBSA exceeding 25% effect the immunological system substantially, we sub-classified patients into two severity groups (TBSA burned <25%; TBSA burned ≥25%).[11]

The ABSI summarizes predictive factors as age, gender, TBSA burned and additive trauma or disease and predicts an increase in patients mortality related to an elevation of the score.[12] Therefore, we compared according to Piatkowski *et al*. the sCD163 levels of the ABSI group <10 to the group of patients with an ABSI ≥ 10 points.[11] The severity of organ dysfunction was compared to the value of sCD163 based on two groups of patients with differing SOFA scores (<10 points; ≥11 points).

All assessments were taken at five predefined time points (admission on ICU, 12, 24, 48 and 120 hours after the burn trauma) for each participant of the study.

Biochemical analysis

Samples were stored in aliquots at -80 °C until analysis. sCD163 was measured with a commercial test kit for research use (BMA Biomedicals, Switzerland) which consists of a three-step non-competitive sandwich assay.

Sealed plates were incubated over night at 20-25 °C. To

perform an antigen detection, the plates were washed three times, softly blotted, and received 200 μl of detection antibody per well and were once again sealed and incubated for 1 h at 37 °C in a humid atmosphere.

The once more washed plates were added with 200 μl diluted peroxidase conjugate (1 in 100) per well for enzyme coupling and incubated for 1 h at 37 °C in a humid atmosphere after sealing.

After the last washing step with the wash buffer, 200 μl freshly prepared tetramethylbenzemidine (TMB) working solution was added per well for the colour reaction. Plates were incubated for exactly 10 min at 20 °C and the reaction was stopped by adding 100 μl stop solution (1N H_2SO_4). Absorbance was read within the following 10 min in a microplate reader at 450 nm, with reference wavelength set to 620 nm.

Means were formed from duplicates. Normal serum sCD163 level in healthy donors was detected with 150 ng/ml (range 50-300 ng/ml, n=8) and intra-assay variation has been declared as determined <5% (n=12) by the manufacturer.

Other parameters and blood counts were assessed by routine laboratory procedures.

Statistics

Nonparametric tests (Mann-Whitney test, Wilcoxon signed rank) were used for comparison of groups, comparison of time points within a group and correlation analyses as indicated. Correlations between different inflammation markers were studied using Pearson bivariate correlation coefficient. Correlations between serum markers and clinical parameters were calculated using Spearman rank order correlation. The null hypothesis was rejected when *P*-value was <0.05. Analyses were performed using Statistical Program for Social Science for Windows (release 15 standard version, SPSS Inc.).

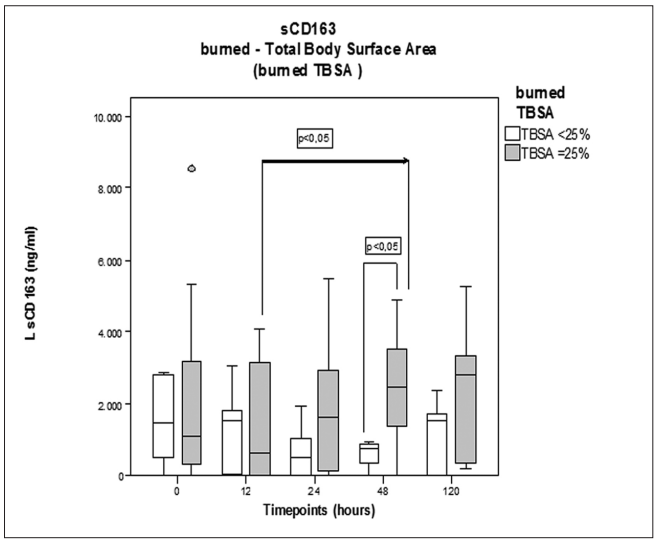

Figure 1: Serum levels of sCD163 (L) in patients with burn trauma in relation to the total body surface area (TBSA) burned group A (white boxes) TBSA <25% (n=8) in comparison with group B (grey boxes) TBSA of 25% or more (n=10)

RESULTS

The concentrations of sCD163 ranged from 48 up to 8531 ng/ml. Compared to the study of Gaini *et al*. these concentrations display a normal range of sCD163 in critically ill patients. All of the critically ill patients included in this study had elevated serum levels of sCD163 compared to the healthy controls documented by Gaini *et al*.[13]

Soluble CD163 (sCD163) in relation to total body surface area burned

Patients were classified in two groups according to TBSA burned. A statistically significant higher level of sCD163 was observed in patients with TBSA burned ≥ 25% (n=10) compared to those with TBSA burned < 25% (n=8) after 48 h (*P*=0.019, Mann-Whitney test). Additionally, patients with TBSA burned > 25% had statistically significant elevated sCD163 levels at 48 h compared to those at 12 h after the burn injury (*P*=0.038, Wilcoxon signed rank) [Figure 1].

Soluble CD163 in relation to abbreviated burn severity index

According to the results for TBSA, sCD163 showed a very similar time-dependent behaviour within the group of patients with an ABSI of 10 points or more (n=7) on admission to the ICU. We were able to detect a significant increase of sCD163 levels in patients with an ABSI ≥ 10 points between the measurements at 12 and 120 h (*P*=0.043, Wilcoxon signed rank). Additionally, there was a decrease of sCD163 among patients with ABSI less than 10 between the time points 12 and 24 h (*P*=0.028, Wilcoxon signed rank) [Figure 2].

Soluble CD163 in relation to initial interleukin-6

Soluble CD163 shows a significant run of the curve according to the initial level of interleukin-6 (IL-6). We found a significant increase of sCD163 in the group of burn patients with an initial IL-6 ≥ 100 pg/ml (n=9) between the measurements at 12 and 120 h (*P*=0.043 (Wilcoxon signed rank)) as well as between 24 and 120 h (*P*=0.043 (Wilcoxon signed rank) [Figure 3].

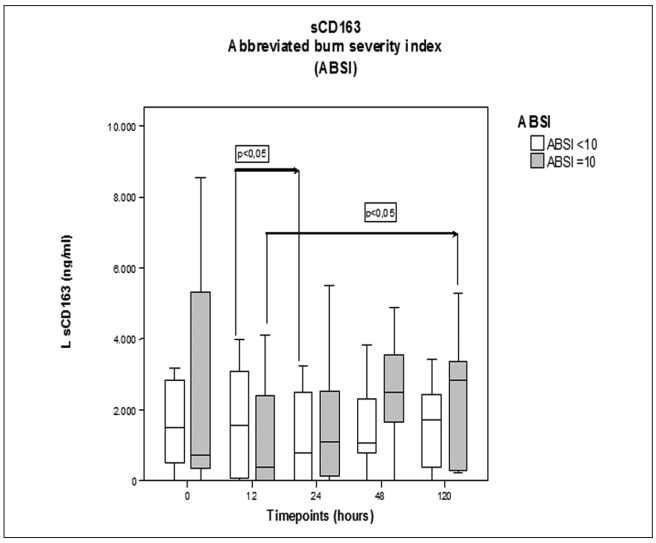

Figure 2: Serum levels of sCD163 (L) in patients with burn trauma in relation to the abbreviated burn severity index (ABSI) group A ABSI <10 (n=11) in comparison with group B ABSI of 10 or more (n=7). Statistical significant differences between groups (Mann-Whitney test) and time points (Wilcoxon signed rank) are labelled under specification of the level of significance

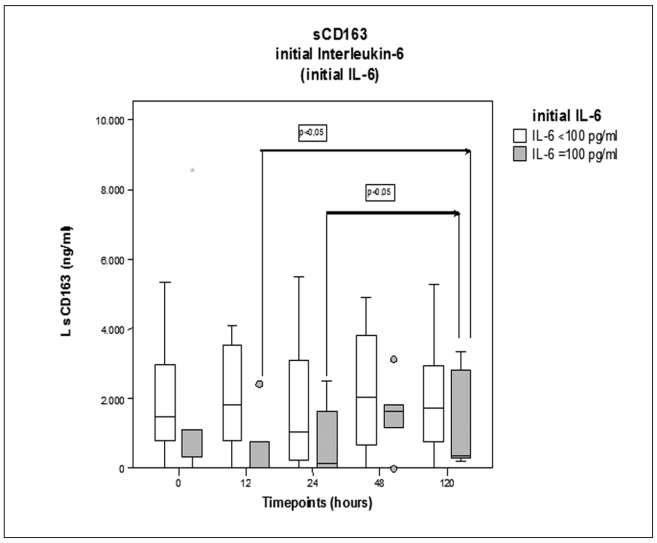

Figure 3: Serum levels of sCD163 (L) in patients with burn trauma in relation to the initial measured level of IL-6 group A initial IL-6 <100 pg/ml (n=9) in comparison with group B initial IL-6 of 100 pg/ml or more (n=9). Statistical significant differences between groups (Mann-Whitney test) and time points (Wilcoxon signed rank) are labelled under specification of the level of significance

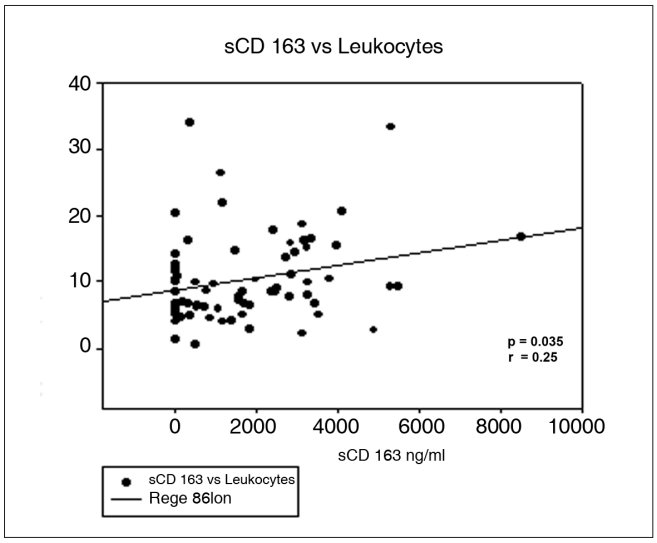

Figure 4: Regression plot between serum level of soluble CD163 and leukocyte count in patients with burn trauma. The Positive linear regression describes a relationship between the tested variables (*P*=0.035, r=0.25)

Table 2: Median SOFA scores

SOFA score	*Mean (range)*
Admission SOFA	6 (0-12)
12 h SOFA	7 (0-12)
24 h SOFA	7.47 (0-13)
48 h SOFA	8.33 (0-15)
120 h SOFA	7.86 (0-16)

Table 3: Correlation of sCD163 in burn patients with calcium, leucocytes, CRP and PCT

Parameter	*Pearson bivariate correlation coefficient*	*P-value*	*N*
Calcium	-0.235	0.048*	71
Leukocytes	0.250	0.035*	71
C-reactive protein	0.157	0.276	50
Procalcitonin	0.116	0.432	48

*Significant values

Soluble CD163 (in relation to commonly used markers of inflammation and calcium

Soluble CD163 was correlated to leukocyte counts and serum calcium concentration [Table 3]. No direct correlation was observed with CRP. No correlation between sCD163 and PCT could be found.

The fragmented analysis of parameter interrelation between sCD163 and leucocytes depending on the predefined time points resulted in a positive correlation between the two parameters [Table 4 and Figure 4]. Furthermore, a negative

Table 4: Correlation between sCD163 serum levels and leucocytes in burn patients on the predefined time points: Admission, 12, 24, 48 and 120 hours (h).

Time point	*Pearson bivariate correlation coefficient*	*P-value*	*N*
Initial	0.191	0.496	18
12 h	0.504	0.066	17
24 h	0.589	0.027*	17
48 h	0.070	0.813	17
120 h	0.567	0.034*	17

*Significant values

correlation between calcium concentrations and sCD163 levels was notable ($P<0.05$; r=-0.235).

Soluble CD163 in relation to sepsis

The statistical analyses of the difference between sCD163 in patients with sepsis compared with burn patients with non-infected SIRS or without any inflammatory signs or infection showed a significantly lower level of the soluble protein in the septic group at 12 h after the injury ($P=0.041$ (Mann-Whitney test)). Furthermore, sCD163 serum concentrations were significantly increased among patients with sepsis between 12 and 120 ($P=0.043$ (Wilcoxon signed rank)), 24 and 48 ($P=0.041$ (Wilcoxon signed rank))as well as 24 and 120 h ($P=0.046$ (Wilcoxon signed rank)) [Figure 5].

Soluble cd163 in relation to severity organ failure assessment score

Patients were assigned to two groups depending on the maximum reached SOFA score during the observed course of time.

Burn patients with a SOFA score from 0 to 10 points within the first 120 h after the injury (group A; n=10) showed a significant decrease of sCD163 levels between 12 and 24 h after burn trauma. In contrast, patients with a SOFA-score of 11 points or more (n=8) showed a significant increase of sCD163 levels between the time points 12 and 120 h after admission [Figure 6 and Table 2]. In order to confirm our hypothesis that higher SOFA scores involve high sCD163 levels, we performed a Spearman rank order correlation, which revealed a positive correlation between SOFA scores and sCD163 levels ($P<0.05$; r= -0.232).

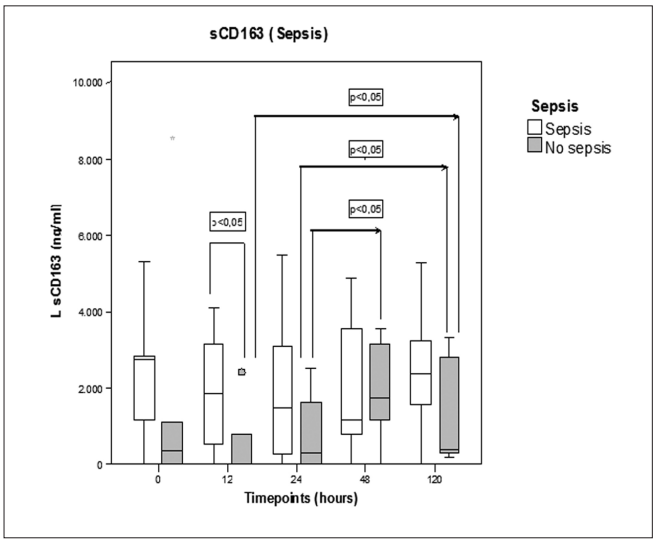

Figure 5: Serum levels of sCD163 (L) in patients with burn trauma in relation to sepsis. Group A burn patients without diagnose of a sepsis (n=12) in comparison with group B patients with sepsis (n=6). Statistical significant differences between groups (Mann-Whitney test) and time points (Wilcoxon signed rank) are labelled under specification of the level of significance

DISCUSSION

Anti-inflammatory response and immunosuppression following burn injury are characterized by opposing cell types and cytokines. The exact sequence of events that result in immunosuppression and lead to sepsis after burn injury, remains yet unknown. However, changes that may affect the immune system include mobilization of mononuclear cells, lymphocytes, and leukocytes, the endocrine system, the arachidonic acid cascade and the cytokine network.[14]

The fact that sCD163 plays a marked role in the processes involving inflammation and macrophage activation leads us to a closer look at the characteristics of septic burn patients with or without multi organ failure and the comparison to the non-septic group.

Several assays have shown that the extracellular part of CD163 (sCD163) is shed into plasma stimulated by mediators of inflammation.[5,6,8] Following an insult like burn trauma the shedding of sCD163 from the surface of monocytes and macrophages into the extracellular space is likely. In this study, we could prove for the first time that sCD163 is found elevated in the serum of burn patients. Furthermore, we were able to expose that 12 h after admission sCD163 levels

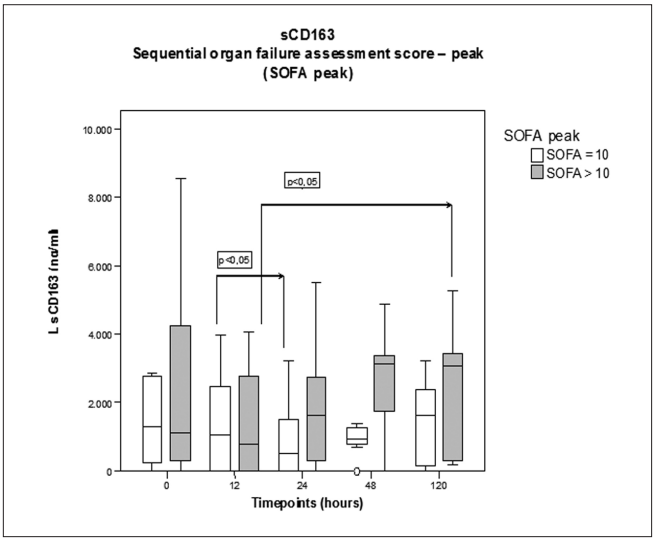

Figure 6: Serum levels of sCD163 (L) in patients with burn trauma in relation to the peak of the SOFA score within the first 120 h after the burn injury. Group A burn patients with a SOFA score (n=10) from 0 to 10 points in comparison with group B patients with a SOFA score of 11 points or more (n=8). Statistical significant differences between groups (Mann-Whitney test) and time points (Wilcoxon signed rank) are labelled under specification of the level of significance

were significantly elevated in patients that developed sepsis [Figure 5] during their course of stay. This could be caused by degradation, elimination or even a massive uptake by T-lymphocytes as described by Högger *et al*.[15,16]

In vitro analyses have shown that soluble CD163 interacts with T-lymphocytes[8] and actively inhibits lymphocyte proliferation.[15,16] Altogether it is apparent that sCD163 leads to depression of the immune capability when lowered as for instance after uptake into lymphocytes.

These results lead to the assumption that sCD163 may have an early predictive value for sepsis and/or multi organ failure after burn trauma since it is known to be a indicator of leukocyte activation.[3] Therefore, high levels of sCD163 are associated with the development of sepsis in burned patients.

This is backed by the comparison of the two different SOFA groups that represent the severity of the patients' organ dysfunction. We were able to observe an increase of sCD163 in the stronger impaired group in contrast to a decrease in the group with less severity of dysfunction in the later phase after burn injury (48 h and later). This may also be based on the connection between sCD163 and organ dysfunction. Further evidence for the connection between sCD163 and MODS/MOF is needed, since this connection may as well be due to the severity of disease. A marker for the severity of disease in burns is the ABSI. Additionally, the ABSI as a predictive scheme for mortality combines factors like age, gender and mode of injury. In patients with burned areas exceeding more than 25% TBSA as well as with an ABSI of 10 points or higher we were able to detect a significant increase of sCD163 after 12 h.

Based on our findings it is possible that there may exist a timeframe beginning at 12 h after burn trauma, when strong alterations of sCD163 become detectable, that could be used in clinical practice as well.

In an *in vitro* analysis, Weaver *et al*. were able to show that the production of IL-6 was followed by an increase of CD163.[17] This effect was reproducible in our study since we observed an increase of sCD163 that followed high initial levels of IL-6. Additionally, a former clinical trial has shown that in patients with bacteraemia sCD163 correlated with IL-6 but not with PCT and CRP levels, which support our findings.[13]

Nevertheless, leukocytes and sCD163 are correlated. The amount of sCD163 can therefore be used as an indicator for the activation of the immunologic system. High sCD163 levels reflect activation and consecutive immune paralysis. Therefore, initially high levels of sCD163 should lead to an awareness of possible septic complications like multiple organ failure.

The specificity of sCD163 remains until now unclear. Several studies have used sCD163 as a marker for bacteriema or activation of the immune system but its role in clinical diagnostics remains unclear. After this first view of sCD163 after burn injury it will be of future interest to achieve definite measured values of this soluble protein after burn trauma. Particular attention should be given to individuals that are reduced in their inflammatory host response to infection by natural conditions like age or pre-existent diseases since the level of sCD163 is low in the elderly, i.e. over 75 years.

CONCLUSION

In this study, we have characterized for the first time the changes of sCD163 levels in the serum of burn patients after extensive burn injury. Patients with high SOFA scores, sepsis and/or MODS/MOF during the time of hospitalization show high levels of sCD163.

In the case of sCD163 we see a high potential for receiving important insight into the inflammatory events during the acute phase after burn trauma. Further studies have to add evidence for an option to intervene in the immune system of burn patients to avoid the formation of sepsis and related complications such as multi organ dysfunction with the goal to achieve a lower mortality rate. sCD163 as an early marker for the severity of the disease could be of great relevance, not only in burn patients but in septic patients as well. In order to further establish if sCD163 can be of significant relevance in the treatment of burns a study with a larger patient group is necessary.

ACKNOWLEDGMENT

We like to thank Dörthe Seidel for her help in this study.

REFERENCES

1. Mosser DM. The many faces of macrophage activation. J Leukoc Biol 2003;73:209-12.
2. Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, *et al*. Identification of the haemoglobin scavenger receptor. Nature 2001;409:198-201.
3. Buechler C, Ritter M, Orso E, Langmann T, Klucken J, Schmitz G.

Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. J Leukoc Biol 2000;67:97-103.
4. Moestrup SK, Moller HJ. CD163. A regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. Ann Med 2004;36:347-54.
5. Droste A, Sorg C, Hogger P. Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family. Biochem Biophys Res Commun 1999;256:110-3.
6. Sulahian TH, Hintz KA, Wardwell K, Guyre PM. Development of an ELISA to measure soluble CD163 in biological fluids. J Immunol Methods 2001;252:25-31.
7. Moller HJ, Peterslund NA, Graversen JH, Moestrup SK. Identification of the hemoglobin scavenger receptor/CD163 as a natural soluble protein in plasma. Blood 2002;99:378-80.
8. Timmermann M, Hogger P. Oxidative stress and 8-iso-prostaglandin F(2alpha) induce ectodomain shedding of CD163 and release of tumor necrosis factor-alpha from human monocytes. Free Radic Biol Med 2005;39:98-107.
9. Hintz KA, Rassias AJ, Wardwell K, Moss ML, Morganelli PM, Pioli PA, *et al*. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. J Leukoc Biol 2002;72:711-7.
10. ACCP/SCCM Consensus Conference Committee. Definition for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992;20:864-74.
11. Piatkowski A, Groger A, Pantel M, Bozkurt A, Fuchs PC, Pallua N. The extent of thermal injury affects fractions of mononuclear cells. Burns 2009;35:256-63.
12. Tobiasen J, Hiebert JM, Edlich RF. The abbreviated burn severity index. Ann Emerg Med 1982;11:260-2.
13. Gaini S, Pedersen SS, Koldkaer OG, Pedersen C, Moestrup SK, Moller HJ. New immunological serum markers in bacteraemia: Anti-inflammatory soluble CD163, but not proinflammatory high mobility group-box 1 protein, is related to prognosis. Clin Exp Immunol 2008;151:423-31.
14. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev 2006;19:403-34.
15. Frings W, Dreier J, Sorg C. Only the soluble form of the scavenger receptor CD163 acts inhibitory on phorbol ester-activated T-lymphocytes, whereas membrane-bound protein has no effect. FEBS lett 2002;526:93-6.
16. Hogger P, Sorg C. Soluble CD163 inhibits phorbol ester-induced lymphocyte proliferation. Biochem Biophys Res Commun 2001;288:841-3.
17. Weaver LK, Pioli PA, Wardwell K, Vogel SN, Guyre PM. Up-regulation of human monocyte CD163 upon activation of cell-surface Toll-like receptors. J Leukoc Biol 2007;81:663-71.

Source of Support: Nil, **Conflict of Interest:** None declared.