Citation	Ingels C, Møller HJ, (2013), Circulating levels of the shed scavenger receptor sCD163 and association with outcome of critically ill patients. J Clin Immunol. 2013 Apr;33(3):619-29.
Archived version	Author manuscript: the content is identical to the content of the published paper, but without the final typesetting by the publisher
Published version	http://dx.doi.org/10.1007/s10875-012-9830-9
Journal homepage	http://link.springer.com/journal/10875
Author contact	greet.vandenberghe@med.kuleuven.be your + 32 (0)16 344021
IR	https://lirias.kuleuven.be/handle/123456789/366322

(article begins on next page)

**Circulating levels of the shed scavenger receptor sCD163
and association with outcome of critically ill patients**

Catherine Ingels¹, Holger J Moller², Troels K Hansen³,
Pieter J Wouters¹, Ilse Vanhorebeek¹, Greet Van den Berghe¹

¹ Clinical Department and Laboratory of Intensive Care Medicine, Division Cellular and Molecular
Medicine, KU Leuven, Herestraat 49, 3000 Leuven, Belgium

² Department of Clinical Biochemistry, Aarhus University Hospital, Norrebrogade 44, DK-8000
Aarhus C, Denmark

³ Immunoendocrine Research Unit, Aarhus University Hospital, Norrebrogade 42-44, DK-8000
Aarhus C, Denmark

Corresponding author:

Prof Greet Van den Berghe, MD, PhD

Clinical Department and Laboratory of Intensive Care Medicine

Division of Cellular and Molecular Medicine

KU Leuven

B-3000 Leuven, Belgium

Phone +32-16-344021 Fax +32-16-344015

E-mail greet.vandenberghe@med.kuleuven.be

26
27 **ABSTRACT**

28
29 **Purpose:** CD163, a scavenger receptor for haptoglobin-hemoglobin complexes, is almost exclusively expressed
30 by monocytes and macrophages and is shedded (as sCD163) by inflammatory stimuli. Thus, high serum levels of
31 sCD163 predicted mortality in certain inflammatory diseases. We investigated baseline levels, time course of
32 sCD163 levels and CD163 gene expression in relation to mortality and clinical complications in a large
33 heterogeneous cohort of critically ill patients.

34 **Methods:** In this pre-planned secondary analysis of two randomized clinical studies, critically ill patients
35 (n=1657) were randomized to “conventional” (insulin administered only for blood glucose levels above 215
36 mg/dL) or “intensive” insulin therapy (glycemia maintained at 80-110 mg/dL) and compared with healthy
37 controls (n=112).

38 **Results:** Upon admission, critically ill patients had 1.6-fold higher sCD163 levels than controls ($P<0.0001$).
39 Long-stay patients (ICU stay >5 days), non-survivors and patients who developed liver dysfunction or kidney
40 injury had higher baseline sCD163 levels. In multivariable analyses, elevated baseline sCD163 levels were
41 independently associated with ICU mortality, liver dysfunction, and time to discharge from ICU and hospital.
42 sCD163 further increased during ICU stay in patients who developed organ dysfunction and in non-survivors.
43 Avoiding hyperglycemia with insulin slightly reduced circulating sCD163 levels. Hepatic CD163 gene
44 expression in patients was higher than in controls ($p=0.002$) and correlated positively with sCD163 levels
45 ($\rho=0.345$, $P<0.0001$).

46 **Conclusions:** This study hallmarks the association of elevated sCD163 with organ dysfunction and adverse
47 outcome of critical illness and may point to the liver as a potential source.

51 **KEYWORDS**

52

53 Critical illness, sCD163, mortality, organ dysfunction, inflammation

54

55

INTRODUCTION

CD163 is a transmembrane glycoprotein scavenger receptor almost exclusively expressed by the monocyte–macrophage cell lineage [1]. Its expression increases during the maturation of monocytes to macrophages, particularly to the M2 or “alternative activation” phenotype, which is induced by glucocorticoids, interleukin-6 (IL-6) and IL-10 [2]. CD163 scavenges haemoglobin-haptoglobin complexes from the circulation and plays an important homeostatic role in the resolution of inflammation [3]. As such, membrane-bound CD163 may be protective in critical illness, where inflammation is a major contributor to morbidity and mortality [4].

Under inflammatory conditions, ectodomain shedding of the CD163 receptor can be induced by lipopolysaccharide (LPS) via Toll-like receptor-4 (TLR-4) activation, by immune complexes cross-linking Fcγ receptors and by oxidative stress mediators (e.g. advanced glycation end products) [5-7]. The physiological role of the soluble receptor (sCD163) remains hitherto unknown. It binds hemoglobin-haptoglobin complexes, but with much lower affinity than the anchored transmembrane receptor [8].

Serum sCD163 levels reflect macrophage activation and have shown to be substantially elevated in hemophagocytic syndromes [9], liver disease [10, 11] and bacteremia/sepsis [12-14]. Moderately elevated levels are seen in chronic and low-grade inflammatory states [15-20]. As recently reviewed, high sCD163 levels predict mortality in a number of diseases, including hemophagocytic syndromes, tuberculosis, chronic kidney disease, bacteremia, liver failure, and the systemic inflammatory response syndrome (SIRS) [21].

To date, the importance of serum sCD163 and the time course of sCD163 release in a broad population of critically ill patients with varying pathology in relation to organ failure and death remain unknown. In the present study, we describe the time course of sCD163 shedding in a mixed population of critically ill adults and assess its predictive value for adverse outcome, as well as its association with development of organ dysfunction.

In addition, we put forward the question whether sCD163 may reflect infection rather than inflammation, for which we studied its relation with bacteremia as outcome variable of severe infection. We hypothesized that admission levels of sCD163 are associated with intensive care unit (ICU) outcome and are lowered by intensive insulin therapy targeting normoglycemia (IIT), which previously showed to exert anti-inflammatory effects [22-24]. Finally, since CD163 is known to be up-regulated on liver macrophages during acute hepatitis [25], and circulating levels of sCD163 are highly increased in both hepatitis and liver failure [10], we further speculated that the liver macrophages may be an important source of sCD163 shedding, which could be up-regulated during critical illness. Therefore, we studied CD163 gene expression in liver biopsies.

METHODS

Patients

This study is a sub-analysis of patients enrolled in two large prospective, randomized, controlled studies assessing the effect of maintenance of normoglycemia with insulin infusion (intensive insulin therapy, IIT) on outcome in a University Hospital surgical and medical ICU [22, 23]. The current substudy is an extension of the pre-planned investigation of potential anti-inflammatory properties of insulin therapy [24]. Strict glycaemic control to 80-110 mg/dL (4.4-6.1 mmol/L) was compared with treatment of hyperglycemia only when rising above 215 mg/dL (conventional insulin therapy, CIT). We previously demonstrated that the effects of IIT on mortality and critical illness-associated complications were most pronounced in prolonged critically ill patients. Therefore, we decided to focus on these ‘long-stayers’ (ICU stay >5 days). However, to exclude selection bias, we also studied a randomly selected cohort of short-stay patients with comparable demographic characteristics and reason for ICU admission as these long-stay patients. The final study population hence consisted of 1657 adult ICU patients (757 surgical and 900 medical ICU patients). Patients in the CIT and IIT groups were comparable for demographic characteristics (Table 1). Mean morning blood glucose levels were significantly lower in IIT (104 ± 21 mg/dL) as compared with CIT patients (150 ± 35 mg/dL, $P < 0.0001$) for which higher doses of insulin were required (63 [41-89] versus 3 [0-31] IU/day, $P < 0.0001$). We included a control group, consisting of healthy volunteers ($n=112$), matched for gender ($P=0.3$), age ($P=0.6$) and BMI ($P=0.6$) to the critically ill patients.

Liver biopsies were harvested immediately postmortem from 131 deceased ICU patients (57 medical and 74 surgical ICU patients) and compared with biopsies from 19 “healthy” patients undergoing major non-complicated elective abdominal surgery for restorative rectal resection, matched for gender ($P=0.9$), age ($P=0.1$) and BMI ($P=0.9$) to the critically ill patients.

Written informed consent was obtained from the volunteers, the patients or next-of-kin. The KU Leuven Institutional Review Board approved the protocols and consent forms (ML1094, ML1820, ML2707). The study has been performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

Biochemical analyses

C-reactive protein (CRP) and markers of organ damage/dysfunction, including creatinine, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and prothrombin time

(PT), were determined by routine clinical chemistry. Daily blood samples had been taken at 6 a.m. during the studies. After centrifugation, serum was kept frozen at -80°C until analysis. We quantified sCD163 levels in serum samples taken on day 1, 5, 10, 15 and on the last day of ICU stay (total no of samples 4,978) using an in-house enzyme-linked immunosorbent assay, essentially as previously described [26, 27]. Briefly, rabbit anti-CD163 (4 mg/L) was coated onto microtitre wells and plates transferred to a BEP-2000 enzyme-linked immunosorbent assay (ELISA)-analyzer (Dade Behring, Eschborn, Germany). Samples (diluted 1:101) were added in duplicates and incubated for 1.5 h at 37°C. Monoclonal anti-CD163 (GHI/61, 3 µg/mL) was then added for 1 h at 37°C, followed by incubation for 1 h at 37°C with horseradish peroxidase-labelled goat anti-mouse antibodies (0.125 µg/mL; Dako, Glostrup, Denmark). The plates were developed with tetramethylbenzidine (TMB) substrate solution (Kem-En-Tec, Taastrup, Denmark.). The assay was calibrated using serum traceable to purified human CD163, with the lowest calibrator being 6.25 µg/L. The inter-assay coefficient of variation on control samples included on each plate (181 runs) was 4.9 % at 1.36 mg/L and 5.8 % at 3.66 mg/L.

CD163 mRNA expression was quantified by real-time PCR. Total RNA was isolated and purified from liver biopsies using Qiazol and the RNeasy mini RNA isolation kit (QIAGEN, Venlo, The Netherlands). Samples were treated with DNase to remove genomic DNA. One microgram of RNA was reverse-transcribed to cDNA using random hexamers. Real-time PCR was performed on the AB StepOnePlus (Applied Biosystems, Foster City, CA) with commercially available gene expression assays for CD163 (Hs00174705_m1) and hypoxanthine-guanine phosphoribosyltransferase (HPRT, 4310890E) from Applied Biosystems. Data for CD163 are expressed normalized to HPRT expression as fold change compared to the median of controls. The presented data were not confounded by treatment with corticosteroids.

Statistical analysis

StatView 5.0.1 (SAS Institute Inc., Cary, NC), JMP9 (SAS) and MedCalc12.1.1 (MedCalc Software, Mariakerke, Belgium) were used for the statistical analyses. Data are presented as mean and standard deviation, median and interquartile range, or proportion and percentages, as appropriate. Variables with a normal distribution were analyzed with Student's t-test, variables with a non-normal distribution with Mann-Whitney U or Kruskal-Wallis test, and proportions with the chi-square test. Associations between variables were calculated with Spearman's correlation coefficient. Repeated-measures ANOVA was used to compare the time course of sCD163 in different subgroups. To further assess the significant univariable associations of baseline sCD163 with ICU outcomes, independent of known risk factors, multivariable logistic regression analysis was performed.

The known classical baseline characteristics and risk factors (gender, age, BMI, history of diabetes or malignancy, medical versus surgical ICU, severity of illness, admission diagnosis, sepsis upon admission, baseline CRP and blood glucose level) were considered, together with the sCD163 serum level upon admission and the randomized intervention. Of this list, the non-collinear factors to enter into the regression models were first identified with stepwise mixed (backward and forward) fit analysis, with probability to leave or enter the fit model set at $P \leq 0.250$. To evaluate independent association of baseline sCD163 with duration of stay in ICU, Cox proportional hazard analysis was performed using the same parameters as described above. The non-survivors were censored beyond the longest staying survivor hereby avoiding bias by competing risks [28]. Receiver-operating characteristic (ROC) curves for baseline levels of sCD163 and for C-reactive protein (CRP, another routinely measured inflammatory marker related to organ failure and mortality of critically ill patients [29-31]), in the prediction of ICU outcome were drawn for the outcomes significant in multivariable analyses and area under the curve (AUC) and corresponding 95% confidence intervals (CI) were calculated. ROC curves were compared with the method of DeLong et al [32] in Medcalc. Two-sided P values below 0.05 were considered significant. The study has a >85% power to demonstrate a 15% reduction in sCD163 on day 5 with IIT.

RESULTS

Association of baseline sCD163 levels with complications of critical illness

Baseline sCD163 levels of critically ill patients (2.13 [1.33-4.27] mg/L) were higher than those of the control group (1.29 [0.94-1.78] mg/L, $P<0.0001$). In univariable analysis, long-stay patients showed higher baseline levels of sCD163 than short-stayers ($P<0.0001$). ICU non-survivors had significantly higher baseline sCD163 levels than survivors (3.46 [1.87-8.82] mg/L vs 1.95 [1.24-3.49] mg/L, $P<0.0001$), irrespective of short- or long-stay (Figure 1a). Similar differences were observed for hospital-mortality (data not shown). Patients with sepsis upon admission had higher sCD163 levels than those without sepsis (2.49 [1.48-4.90] mg/L vs 1.93 [1.25-3.69] mg/L, $P<0.0001$). However, no clear differences in baseline sCD163 levels were observed between patients who later developed bacteremia as compared to those who did not (Figure 1a). Both short- and long-stay patients who developed cholestatic liver dysfunction, defined as a peak plasma bilirubin exceeding 3 mg/dL [28], had higher baseline sCD163 levels than those who did not (Figure 1a). Similar associations were observed for several other serum markers of hepatic injury, including ALT, AST, LDH, and PT (all $P<0.0001$, data not shown). In the total patient population of our study, levels of baseline sCD163 were higher in patients who developed new kidney injury, defined as a doubling of baseline serum creatinine (2.27 [1.78-4.49] mg/L vs 2.09 [1.28-4.21] mg/L, $P=0.0005$). This effect appeared attributable to the higher levels in short-stay patients who developed kidney injury, since sCD163 levels in long-stay patients were comparable whether they developed new kidney injury or not (Figure 1a).

In multivariable regression analysis correcting for all other baseline risk factors, elevated sCD163 levels on admission were independently associated with ICU mortality and occurrence of liver dysfunction, whereas such impact for new acute kidney injury was less clear ($P=0.08$, Table 2). Sensitivity analyses, in which we included each variable separately together with the baseline sCD163 level, confirmed the robustness of our models as none of the variables changed the odds ratio of sCD163 with more than 10% (data not shown). Likewise, in Cox proportional hazard analysis correcting for all baseline risk factors, high baseline sCD163 levels were independently associated with prolonged time to alive ICU and hospital discharge (Table 3).

Baseline sCD163 and CRP levels weakly correlated ($\rho=0.178$, $P<0.0001$). Performance of baseline sCD163 levels in prediction of ICU mortality, liver dysfunction and renal failure was compared with that of baseline CRP levels using ROC curve analysis. sCD163 was superior to CRP in the prediction of mortality (AUC of 0.68

(0.65-0.70) versus 0.57 (0.54-0.59), $P<0.0001$) and especially in the prediction of liver dysfunction (0.73 (0.70-0.75) versus 0.54 (0.51-0.56), $P<0.0001$) (Figure 2).

As it had been demonstrated previously that sCD163 lost its power in predicting survival after pneumococcal bacteremia when patients were older than 75 years [14], we performed similar analysis for this older subgroup. Patients older than 75 years ($n=371$, 22.4%) had slightly, non-significantly lower baseline levels of sCD163 (2.01 [1.37-3.36] mg/L) than patients younger than 75 years (2.17 [1.30-4.63] mg/L, $P=0.06$). Nevertheless, in these older patients higher baseline sCD163 levels were still associated with ICU and hospital mortality, longer time in ICU and liver dysfunction, but not with new kidney failure or the acquisition of bacteremia (data not shown).

Time course of sCD163 levels in the circulation and association with complications of critical illness

Both in short- and long-stay patients, last day sCD163 levels were higher than the corresponding baseline levels ($P<0.0001$). In the long-stay patients sCD163 levels gradually increased over time from baseline to days 5, 10 and 15 (data not shown). This rise in sCD163 with ICU stay was notably more pronounced in non-survivors than survivors, and in patients who developed cholestatic liver dysfunction or new kidney injury than in those who did not (Figure 1b), superimposed on the already elevated levels at baseline. In contrast, the time course of sCD163 levels throughout ICU stay was not different in patients who acquired bacteremia as compared to those who did not.

Effect of intensive insulin therapy on sCD163

Baseline sCD163 levels were comparable for patients in the CIT and IIT groups (Figure 3). IIT slightly blunted the further rise in sCD163 levels with time in ICU (Figure 3). The effect of IIT was only present among the survivors (e.g. sCD163 levels on day 5 3.81 [2.45-7.15] mg/L in CIT vs 3.42 [2.05-6.13] mg/L in IIT group, $P=0.009$) but not in non-survivors (e.g. sCD163 on day 5 6.48 [2.81-11.17] mg/L in CIT vs 5.93 [3.13-11.89] mg/L in IIT, $P=0.92$).

CD163 gene expression

The CD163 mRNA levels in liver samples harvested from (non-surviving) critically ill patients (1.93 (1.15-3.50)), irrespective of insulin therapy, were approximately twice those of healthy controls (1.01 (0.58-1.38)),

221 $P=0.002$) (Figure 4). sCD163 serum levels on the day of biopsy correlated with the respective gene expression
222 levels ($\rho=0.345$, $P<0.0001$).

223

224

225

DISCUSSION

We demonstrated that elevated sCD163 levels are strongly associated with several adverse outcomes in a large, heterogeneous ICU patient population. sCD163 levels in critically ill patients were elevated above healthy control levels from baseline onwards and remained high until the last day in ICU. Especially patients who did not survive or patients who developed liver dysfunction or new kidney injury had higher baseline levels as compared to patients without such adverse outcomes. This was accentuated with a remarkably stronger further rise in sCD163 in these patients throughout ICU stay. Baseline elevated sCD163 was associated with ICU mortality and development of organ dysfunction, in particular liver dysfunction, even after correction for baseline risk factors and severity of illness. Likewise, such high levels appeared an independent risk factor for prolonged time to alive discharge from ICU and hospital. In contrast with previous findings for patients with pneumococcal sepsis [14], older age did not compromise the predictive power of sCD163 in this large, heterogeneous study population. Increased hepatic expression of CD163 may have contributed to the elevated levels of the soluble receptor in the circulation. Preventing hyperglycemia with intensive insulin therapy only slightly attenuated sCD163 levels, but did not affect hepatic CD163 gene expression studied in non-survivors. This effect is probably of minor clinical importance.

In this large, unselected and heterogeneous patient population in the ICU, we observed a strong association between high sCD163 levels and lethal outcome. Indeed, baseline sCD163 levels were higher in non-survivors than in survivors and remained independently associated with mortality in multivariable analysis. sCD163 performed better than CRP in ROC analysis, although the AUC was still relatively low. This confirmed the observations in small sets of selected patients, such as those with acute liver failure, in whom sCD163 levels were higher in non-survivors than survivors and, better than CRP, predicted fatal disease outcome [14, 34, 35]. Furthermore, sCD163 levels progressively increased with time in ICU, continuing to rise in non-survivors whereas they stabilized earlier in survivors. Such a gradual increase has also been observed in non-survivors of fulminant hepatic failure [10]. Elevated upon admission sCD163 levels were also independently associated with later development of liver dysfunction, whereas the relation with kidney dysfunction was less clear. Moreover, the same pattern of progressively increasing sCD163 with time in ICU was seen in patients who developed liver dysfunction or renal failure as opposed to patients without organ dysfunction, in whom the sCD163 levels stabilized.

The relation between increased sCD163 and the development of liver dysfunction (increased bilirubin, ALT, AST, decreased PT) during critical illness is of particular interest. Kupffer cells, the liver macrophages, are

responsible for the initiation of the pro-inflammatory response after liver injury [35] and are involved in the pathogenesis of acute liver failure [35, 36]. CD163 is up-regulated on liver macrophages during acute hepatitis [25], and circulating levels of sCD163 are highly increased in both hepatitis and liver failure [10]. Quantitative mRNA analysis on post-mortem liver biopsies in the mixed population of critically ill patients of the present study showed that liver CD163 mRNA expression was highly up-regulated as compared to controls and correlated with circulating sCD163 levels. Therefore, the liver Kupffer cells likely are a source of sCD163 during critical illness.

CD163 is shed from Kupffer cells and other macrophages by protease activity. We recently showed that TNF- α -converting enzyme (TACE, also known as ADAM17), which is responsible for the shedding of active TNF- α , also cleaves CD163 [38]. This suggests that common factors are responsible for part of the increase in both TNF- α and sCD163 seen in inflammatory diseases. One such factor may be bacterial products such as LPS. Intravenous administration of LPS to healthy volunteers has been shown to induce a rapid increase in sCD163 as well as TNF- α in plasma via TLR-4-mediated activation of TACE [38, 39]. In this respect, sCD163 is a result of pro-inflammatory actions. However, the expression level of CD163 on the macrophages also influences the level of sCD163. Upon shedding, the macrophage expression of CD163 is quickly restored and even increased [39]. Furthermore, the expression level is strongly induced by anti-inflammatory cytokines like IL-10 [2]. These cytokines are part of counter-acting anti-inflammatory mechanisms that start the process of healing and repair [36]. The highest levels of sCD163 are therefore expected, and actually occur, in situations of overt counter-regulatory processes that, for some reason, remain unable to dampen a strong pro-inflammatory response. Hemophagocytic syndrome, acute liver failure, and severe sepsis are type-examples of such situations [4, 9]. The membrane-bound CD163 receptor itself has anti-inflammatory properties through scavenging of plasma hemoglobin and products formed from breakdown of toxic heme [3, 36, 40, 41]. CD163 up-regulation to this end may be seen as a macrophage adaptation to inflammation, stimulating counter-regulatory processes [4]. In sepsis, Kupffer cells along with bone marrow macrophages have been shown to strongly express CD163 and to be hemophagocytic, actively ingesting erythrocytes and other blood cells [4]. Furthermore, a post-mortem study of critically ill patients showed macrophage hemophagocytosis in the bone marrow of 65% of cases independent of the underlying disease [42].

Patients with an upon-admission sepsis diagnosis presented with higher baseline sCD163 levels than those without sepsis, but baseline levels of sCD163 were not associated with subsequent acquisition of bacteremia. This further points to sCD163 being a marker of inflammation rather than of infection. These data are in

agreement with previous findings in which sCD163 was not able to discriminate between infected and non-infected patients and performed poorer than CRP as an infection marker [43, 44].

Sustained inflammation can lead to renal failure (as part of the multiple organ dysfunction syndrome) and, vice versa, renal failure fuels the inflammatory response [45-47]. It is therefore not surprising that sCD163 in our study kept increasing in patients who developed kidney dysfunction. Increased levels of sCD163 have previously been reported for chronic kidney disease [48]. Elevated sCD163 related to increased mortality, but was not associated with glomerular filtration rate suggesting that decreased removal by the kidney is not the cause of the increasing serum levels. This is also supported by the large size (130 kDa) of sCD163, which speaks against removal by the kidneys or filtering during dialysis. However, in states of acute kidney injury a fraction of sCD163 may be excreted in the urine, as recently described [49], but this would rather tend to lower serum sCD163. A large part of sCD163 may be removed by the macrophages themselves, e.g. by mechanisms involving weak binding of sCD163 to haptoglobin-hemoglobin-complexes [8]. However, if a fraction of sCD163 is cleared directly by the hepatocytes, it is a possibility that decreasing liver function with decreased plasma clearance may also add to the increasing sCD163 levels seen in these patients. To date, very little is known about how sCD163 is cleared from the circulation.

Like patients with diabetes, critically ill patients develop hyperglycemia and insulin resistance, as part of the stress response [50]. Hyperglycemia further stimulates oxidative stress and promotes inflammation [51]. Avoiding hyperglycemia with intensive insulin therapy may improve survival of critically ill patients, reduce organ failure and attenuate the inflammatory response [22-24, 52]. As hypothesized, intensive insulin therapy reduced, albeit modestly, sCD163 levels after a few days of treatment, consistent with attenuation of inflammation.

It has to be emphasized that this study only shows associations without providing evidence for possible causal mechanisms. Notwithstanding this limitation, this study adds support to the role of sCD163 as a risk factor for poor outcome in different inflammatory states and possibly as a marker of disease severity in critical illness. More research, however, is needed to improve our insight in the physiology and time course of sCD163 release and relation with inflammation, hyperglycemia, development of organ damage and fatal outcome in critical illness.

CONCLUSIONS

Circulating sCD163 levels are elevated during critical illness with the liver likely being a source. This study highlighted the association of high sCD163 levels with organ damage and mortality in critical illness and thus adds to the growing evidence showing increased sCD163 to be an important risk factor in various diseases. Maintaining normoglycemia with insulin therapy slightly lowered sCD163 levels, which underscores the anti-inflammatory effects of the therapy.

APPENDIX

LIST OF ABBREVIATIONS

ADAM17: a disintegrin and metalloprotease domain 17, ALT: alanine aminotransferase, AST: aspartate aminotransferase, AUC: area under the curve, BMI: body mass index, CI: confidence interval, CIT: conventional insulin therapy, CRP: C-reactive protein, HPRT: hypoxanthine-guanine phosphoribosyltransferase, ICU: intensive care unit, IIT: intensive insulin therapy, IQR: interquartile range, LDH: lactate dehydrogenase, LPS: lipopolysaccharide, PT: prothrombin time, ROC: receiver operating characteristic curve, SIRS: systemic inflammatory response syndrome, SD: standard deviation, TACE: TNF- α converting enzyme, TLR: Toll-like receptor, TNF- α : tumor-necrosis factor α .

ACKNOWLEDGEMENTS

We thank Kirsten Bank Petersen for excellent technical assistance.

CI received a Doctoral Fellowship of the Clinical Research Fund of the University Hospitals Leuven. GVdB by the University of Leuven, receives long-term structural research financing via the Methusalem program, funded by the Flemish Government.

COMPETING INTERESTS

HM and Aarhus University have received royalties from IQ-products, the Netherlands.

The other authors declare that they have no competing interest.

REFERENCES

1. Maniecki MB, Møller HJ, Moestrup SK, Møller BK. CD163 positive subsets of blood dendritic cells: the scavenging macrophage receptors CD163 and CD91 are coexpressed on human dendritic cells and monocytes. *Immunobiology* 2006;211:407-17.
2. Ritter M, Buechler C, Langmann T, Orso E, Klucken J, Schmitz G. Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and anti-inflammatory stimuli. *J Leukoc Biol* 2000;67:97-103.
3. Fabrick B, Dijkstra C, Van den Berg T. The macrophage scavenger receptor CD163. *Immunobiology* 2005; 210:153-160.
4. Schaer DJ, Schaer CA, Schoedon G, Imhof A, Kurrer MO. Hemophagocytic macrophages constitute a major compartment of heme oxygenase expression in sepsis. *Eur J Haematol* 2006;77:432-6.
5. Sulahian TH, Pioli PA, Wardwell K, Guyre PM. Cross-linking of FCgammaR triggers shedding of the hemoglobin-haptoglobin scavenger receptor CD163. *J Leukoc Biol* 2004;76:271-7.
6. 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.
7. Van Gorp H, Delputte P, Nauwynck H. Scavenger receptor CD163, a jack-of-all-trades and potential target for cell-directed therapy. *Mol Immunol* 2010;47:1650-60.
8. Møller HJ, Nielsen MJ, Maniecki MB, Madsen M, Moestrup SK. Soluble macrophage-derived CD163: a homogenous ectodomain protein with dissociable haptoglobin-hemoglobin binding. *Immunobiology* 2010; 215:406-12.
9. Schaer DJ, Schleiffenbaum B, Kurrer M, Imhof A, Bächli E, Fehr J, Møller HJ, Moestrup SK, Schaffner A. Soluble hemoglobin-haptoglobin scavenger receptor CD163 as a lineage specific marker in the reactive hemophagocytic syndrome. *Eur J Haematol* 2005;74:6-10.
10. Hiraoka A, Horiike N, Akbar SM, Michitaka K, Matsuyama T, Onji M. Soluble CD163 in patients with liver diseases: very high levels of soluble CD163 in patients with fulminant hepatic failure. *J Gastroenterol* 2005;40:52-6.

375 11. Holland-Fischer P, Grønbaek H, Sandahl TD, Moestrup SK, Riggio O, Ridola L, Aagaard NK, Møller HJ,
376 Vilstrup H. Kupffer cells are activated in cirrhotic portal hypertension and not normalised by TIPS. Gut
377 2011;60:1389-93.

378 12. Møller HJ, Peterslund NA, Graversen JH, Moestrup SK. Identification of the hemoglobin scavenger
379 receptor/CD163 as a natural soluble protein in plasma. Blood 2002;99:378-80.

380 13. Weiss M, Schneider EM. Soluble CD163: an age-dependent, anti-inflammatory biomarker predicting
381 outcome in sepsis. Crit Care Med 2006;34:2682-83.

382 14. Møller HJ, Moestrup SK, Weis N, Wejse C, Nielsen H, Pedersen SS, Attermann J, Nexø E, Kronborg G.
383 Macrophage serum markers in pneumococcal bacteremia : prediction of survival by soluble CD163. Crit Care
384 Med 2006;34:2561-6.

385 15. Bleesing J, Prada A, Siegel DM, Villanueva J, Olson J, Ilowite NT, Brunner HI, Griffin T, Graham TB,
386 Sherry DD, Passo MH, Ramanan AV, Filipovich A, Grom AA. The diagnostic significance of soluble CD163
387 and soluble interleukin-2 receptor alpha-chain in macrophage activation syndrome and untreated new-onset
388 juvenile idiopathic arthritis. Arthritis Rheum 2007;56:3877-8.

389 16. Baeten D, Demetter P, Cuvelier CA, Kruithof E, Van Damme N, De Vos M, Veys EM, De Keyser F.
390 Macrophages expressing the scavenger receptor CD163: a link between immune alterations of the gut and
391 synovial inflammation in spondyloarthritis. J Pathol 2002;196:343-50.

392 17. Aristoteli LP, Møller HJ, Bailey B, Moestrup SK, Kritharides L. The monocytic lineage specific soluble
393 CD163 is a plasma marker of coronary atherosclerosis. Atherosclerosis 2006;184:342-7.

394 18. Fabrick BO, Møller HJ, Vloet RP, van Winsen LM, Hanemaaijer R, Teunissen CE, Uitdehaag BM, van den
395 Berg TK, Dijkstra CD. Proteolytic shedding of the macrophage scavenger receptor CD163 in multiple sclerosis.
396 J Neuroimmunol 2007;187:179-86.

397 19. Asleh R, Marsh S, Shikrut M, Binah O, Guetta J, Lejbkowitz F, Enav B, Shehadeh N, Kanter Y, Lache O,
398 Cohen O, Levy NS, Levy AP. Genetically determined heterogeneity in hemoglobin scavenging and
399 susceptibility to diabetic cardiovascular disease. Circ Res 2003;92:1193-200.

400 20. Philippidis P, Athanasiou T, Nadra I, Ashrafian H, Haskard DO, Landis RC, Taylor KM. Anti-inflammatory
401 haemoglobin scavenging monocytes are induced following coronary artery bypass surgery. Eur J Cardiothorac
402 surg 2010;37:1360-6.

403 21. Moller HJ. Soluble CD163. *Scand Clin J Lab Invest* 2012;72:1-13.

404 22. Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande
405 P, Lauwers P, Bouillon R: Intensive insulin therapy in critically ill patients. *N Engl J Med* 2001;345:1359-67.

406 23. Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E,
407 Bobbaers H, Bouillon R. Intensive insulin therapy in the medical ICU. *N Engl J Med* 2006;354:449-61.

408 24. Hansen TK, Thiel S, Wouters PJ, Christiansen JS, Van den Berghe G. Intensive insulin therapy exerts
409 antiinflammatory effects in critically ill patients and counteracts the adverse effect of low mannose-binding
410 lectin levels. *J Clin Endocrinol Metab* 2003;88:1082-8.

411 25. Hiraoka A, Horiike N, Akbar SM, Michitaka K, Matsuyama T, Onji M. Expression of CD163 in the liver of
412 patients with viral hepatitis. *Pathol Res Pract* 2005;201:379-84.

413 26. Moller H, Hald K, Moestrup S. Characterization of an enzyme-linked immunosorbent assay for soluble
414 CD163. *Scand J Clin Lab Invest* 2002;62:293-9.

415 27. Møller HJ. Biological variation of soluble CD163. *Scand J Clin Lab Invest* 2003;63:15-21.

416 28. Casaer MP, Mesotten D, Hermans G, Wouters PJ, Schetz M, Meyfroidt G, Van Cromphaut S, Ingels C,
417 Meersseman P, Muller J, Vlasselaers D, Debaveye Y, Desmet L, Dubois J, Van Assche A, Vanderheyden S,
418 Wilmer A, Van den Berghe G. Early versus late parenteral nutrition in critically ill adults. *N Engl J Med*
419 2011;365:506-17.

420 29. Pepys M, Hirschfield G. C-reactive protein: a critical update. *J Clin Invest* 2003;111:1805-12.

421 30. Lobo SM, Lobo FR, Bota DP, Lopes-Ferreira F, Soliman HM, Mélot C, Vincent JL. C-reactive protein levels
422 correlate with mortality and organ failure in critically ill patients. *Chest* 2003;123:2043-9.

423 31. Wang F, Pan W, Pan S, Wang S, Ge Q, Ge J. Usefulness of N-terminal pro-brain natriuretic peptide and C-
424 reactive protein to predict ICU mortality in unselected medical ICU patients: a prospective, observational study.
425 *Crit Care* 2011;15(1):R42

426 32. DeLong ER, DeLong DM, Clarke-Pearson DL (1988): Comparing the areas under two or more correlated
427 receiver operating characteristic curves: a nonparametric approach. *Biometrics* 44:837-845

428 33. Mesotten D, Wauters J, Van den Berghe G, Wouters PJ, Milants I, Wilmer A. The effect of strict blood
429 glucose control on biliary sludge and cholestasis in critically ill patients. *J Clin Endocrinol Metab*
430 2009;94:2345-52.

431 34. Gaiini S, Pedersen SS, Koldkaer OG, Pedersen C, Moestrup SK, Møller HJ. New immunological serum
432 markers in bacteraemia: anti-inflammatory soluble CD163, but not proinflammatory high mobility group-box 1
433 protein, is related to prognosis. *Clin Exp Immunol* 2008;151:423-31.

434 35. Møller HJ, Grønbaek H, Schiødt FV, Holland-Fischer P, Schilsky M, Munoz S, Hassanein T, Lee WM; U.S
435 Acute Liver Failure Study Group. Soluble CD163 from activated macrophages predicts mortality in acute liver
436 failure. *J Hepatol* 2007;47:671-6.

437 36. Possamai LA, Antoniadou CG, Anstee QM, Quaglia A, Vergani D, Thursz M, Wendon J. Role of monocytes
438 and macrophages in experimental and human acute liver failure. *World J Gastroenterol* 2010;16:1811-9

439 37. Rolando N, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory
440 response syndrome in acute liver failure. *Hepatology* 2000;32:734-9.

441 38. Etzerodt A, Maniecki MB, Møller K, Møller HJ, Moestrup SK. Tumor necrosis factor α -converting enzyme
442 (TACE/ADAM17) mediates ectodomain shedding of the scavenger receptor CD163. *J Leukoc Biol*
443 2010;88:1201-5.

444 39. Hintz KA, Rassias AJ, Wardwell K, Moss ML, Morganelli PM, Pioli PA, Givan AL, Wallace PK, Yeager
445 MP, Guyre PM. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the
446 monocyte hemoglobin scavenger receptor CD163. *J Leukoc Biol* 2002;72:711-7.

447 40. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and
448 extracellular plasma hemoglobin: a novel mechanism of human disease. *JAMA* 2005;293:1653-62.

449 41. Otterbein LE, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties
450 of heme. *Trends Immunol* 2003;24:449-55.

451 42. Strauss R, Neureiter D, Westenburger B, Wehler M, Kirchner T, Hahn EG. Multifactorial risk analysis of
452 bone marrow histiocytic hyperplasia with hemophagocytosis in critically ill medical patients - a postmortem
453 clinicopathologic analysis. *Crit Care Med* 2004;32:1316-21.

43. Knudsen TB, Larsen K, Kristiansen TB, Møller HJ, Tvede M, Eugen-Olsen J, Kronborg G. Diagnostic value of soluble CD163 serum levels in patients suspected of meningitis: comparison with CRP and procalcitonin. *Scand J Infect Dis* 2007;39:542-53.
44. Gaïni S, Koldkjaer O, Pedersen SS, Pedersen C, Moestrup SK, Møller HJ. Soluble haemoglobin scavenger receptor (sCD163) in patients with suspected community-acquired infections. *APMIS* 2006;114:103-11.
45. Himmelfarb J. Linking oxidative stress and inflammation in kidney disease: which is the chicken and which is the egg? *Sem Dial* 2004;17:449-54.
46. Doi K, Leelahavanichkul A, Yuen PS, Star RA. Animal models of sepsis and sepsis-induced kidney injury. *J Clin Invest* 2009;119: 2868-78.
47. Faubel S. Acute kidney injury and multiple organ dysfunction syndrome. *Minerva Urol Nefrol* 2009;61:171-88.
48. Axelsson J, Møller HJ, Witas P, Qureshi AR, Carrero JJ, Heimbürger O, Bárány P, Alvestrand A, Lindholm B, Moestrup SK, Stenvinkel P. Changes in fat mass correlate with changes in soluble sCD163, a marker of mature macrophages, in patients with CKD. *Am J Kidney Dis* 2006;48:916-25.
49. Su L, Feng L, Jiang Z, Li M, Xiao K, Yan P, Jia Y, Feng D, Liu C, Xie L. Diagnostic value of urine sCD163 levels for sepsis and relevant acute kidney injury: a prospective study. *BMC Nephrol* 2012;13:123
50. Losser M-R, Damoiseil C, Payen D. Bench-to-bedside review: glucose and stress conditions in the intensive care unit. *Crit Care* 2010;14:231-42.
51. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414: 813-20.
52. Vlasselaers D, Milants I, Desmet L, Wouters PJ, Vanhorebeek I, van den Heuvel I, Mesotten D, Casaer MP, Meyfroidt G, Ingels C, Muller J, Van Cromphaut S, Schetz M, Van den Berghe G. Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. *Lancet* 2009;373:547-56.

TABLES

Table 1: Characteristics of the patients

	CIT (n=841)	IIT (n=816)	P
Baseline characteristics			
Demography and anthropometry			
Gender (male) (no., %)	553 (66)	532 (65)	0.8
Age (yr) (mean $\pm$ SD)	62.8 $\pm$ 15.8	62.7 $\pm$ 15.7	0.7
BMI (kg/m ²) (mean $\pm$ SD)	24.9 $\pm$ 5.2	25.4 $\pm$ 5.2	0.03
Medical history			
Diabetes (no., %)	116 (14)	108 (13)	0.7
Malignancy (no., %)	210 (25)	213 (26)	0.6
Underlying illness			
Medical ICU/surgical ICU (no., %)	455/386 (54/46)	445/371 (55/45)	0.9
Diagnostic categories (no.) ^a			
Cardiovascular	172	170	0.8
Abdominal	148	127	0.3
Respiratory	277	286	0.4
Other	244	233	0.8
TISS-28 (mean $\pm$ SD)	34 (9)	34 (9)	0.4
Sepsis upon admission (no., %) ^b	387 (46)	405 (50)	0.1
CRP upon admission (mg/dL) (median – IQR)	113 [52-215]	116 [52-211]	0.8
Blood glucose upon admission (mg/dL) (mean $\pm$ SD)	155 $\pm$ 60	154 $\pm$ 60	0.6
Outcome			
ICU mortality (no., %)	200 (24)	149 (18)	0.006
Days in ICU (median - IQR)	7 [3-16]	7 [3-14]	0.03
New kidney injury (baseline creatinine * 2) (no., %)	97 (12)	50 (6)	<0.0001
Hyperbilirubinemia (peak >3 mg/dL) (no., %)	225 (27)	189 (23)	0.09
Bacteremia (no., %)	101 (12)	75 (9)	0.06

CIT: conventional insulin therapy, IIT: intensive insulin therapy, CRP: C-reactive protein, TISS-28: Therapeutic Intervention Scoring System, 1 mg/dL glucose = (1/18) mmol/L. ^a Diagnostic categories according to primary failing organ system. Cardiovascular: Cardiac disease, cardiac or complicated aortic surgery; Abdominal: gastrointestinal disease or hepatic disease, complicated abdominal surgery or peritonitis; Respiratory: respiratory disease, complications of pulmonary or esophageal surgery. ^b Sepsis upon admission criteria per definition could not be applied for trauma patients and patients who underwent cardiac surgery (164 patients from the conventional and 149 from the intensive insulin therapy group were hence excluded); percentages are expressed relative to all patients.

Table 2: Multivariable analyses with baseline sCD163 levels in relation to outcome

	Odds ratio (95% confidence interval)	<i>P</i>
1. ICU mortality		
Baseline sCD163 level (per mg/l added)	1.080 (1.051-1.109)	<0.0001
Age (per year added)	1.011 (1.002-1.021)	0.02
BMI (per kg/m ² added)	0.962 (0.935-0.989)	0.006
APACHE (per unit added)	1.063 (1.048-1.079)	<0.0001
History of malignancy	1.769 (1.322-2.369)	<0.0001
Diagnostic group : cardiovascular	1.717 (1.014-2.908)	0.04
Diagnostic group : respiratory	1.904 (1.242-2.921)	0.003
Diagnostic group : other	2.071 (1.342-3.197)	0.001
Sepsis on admission	1.365 (1.035-1.801)	0.03
Randomization to IIT	0.667 (0.511-0.869)	0.003
Admission blood glucose (per mg/dl added)	1.003 (1.001-1.005)	0.01
2. Cholestatic liver dysfunction		
Baseline sCD163 level (per mg/l added)	1.184 (1.144-1.225)	<0.0001
Age (per year added)	0.988 (0.979-0.996)	0.005
APACHE (per unit added)	1.042 (1.024-1.060)	<0.0001
History of diabetes	1.621 (1.084-2.423)	0.02
Diagnostic group : cardiovascular	0.667 (0.441-1.008)	0.05
Diagnostic group : respiratory	0.201 (0.135-0.300)	<0.0001
Diagnostic group : other	0.507 (0.356-0.722)	0.0002
Sepsis on admission	1.436 (1.097-1.880)	0.008
Randomization to IIT	0.837 (0.650-1.077)	0.2
Admitted to surgical ICU	2.143 (1.468-3.130)	<0.0001
3. New kidney injury		
Baseline sCD163 level (per mg/l added)	1.028 (0.999-1.059)	0.06
Diagnostic group : cardiovascular	3.839 (1.851-7.964)	0.0003
Diagnostic group : respiratory	2.059 (1.079-3.928)	0.03
Diagnostic group : other	1.624 (0.833-3.168)	0.2
Sepsis on admission	1.514 (1.003-2.285)	0.05
Randomization to IIT	0.545 (0.368-0.807)	0.002
Admission blood glucose (per mg/dl added)	1.002 (1.000-1.005)	0.08
Baseline CRP (per mg/ml added)	1.002 (1.000-1.003)	0.08

Odds ratios and 95% confidence intervals are shown after correction for classical baseline characteristics and risk factors, as well as the randomized intervention. All classical baseline characteristics and risk factors were considered (gender, age, BMI, history of diabetes, history of malignancy, admission to medical or surgical ICU, severity of illness as evaluated by the APACHE-II score, admission diagnosis, sepsis upon admission, admission CRP level, admission glucose level). The actual variables to be entered in the model were identified with stepwise mixed fit analysis. The

498 variables that were not maintained for entering into the model are not listed. For the diagnostic groups,
499 comparisons were made with the abdominal group.
500

Table 3: Cox proportional hazard analysis for association of baseline sCD163 with time in ICU

	Hazard ratio (95% confidence interval)	<i>P</i>
1. Time to discharge from ICU		
Baseline sCD163 level (per mg/l added)	0.946 (0.930-0.962)	<0.0001
Gender (male)	1.054 (0.930-1.194)	0.4
Age (per year added)	0.998 (0.994-1.002)	0.4
BMI (per kg/m2 added)	1.004 (0.992-1.016)	0.6
APACHE (per unit added)	0.970 (0.962-0.978)	<0.0001
History of diabetes	1.188 (0.991-1.425)	0.06
History of malignancy	0.899 (0.778-1.038)	0.1
Diagnostic group : cardiovascular	0.775 (0.621-0.967)	0.02
Diagnostic group : respiratory	0.721 (0.602-0.864)	0.0004
Diagnostic group : other	0.696 (0.581-0.833)	<0.0001
Sepsis on admission	0.825 (0.727-0.936)	0.003
Randomization to IIT	1.181 (1.048-1.330)	0.06
Admitted to surgical ICU	0.842 (0.715-0.990)	0.04
Admission blood glucose (per mg/dl added)	0.999 (0.998-1.000)	0.03
Baseline CRP (per mg/ml added)	0.999 (0.998-0.999)	0.0002
2. Time to discharge from hospital		
Baseline sCD163 level (per mg/l added)	0.942 (0.924-0.961)	<0.0001
Gender (male)	1.101 (0.961-1.260)	0.2
Age (per year added)	0.992 (0.988-0.996)	0.0002
BMI (per kg/m2 added)	1.015 (1.002-1.027)	0.02
APACHE (per unit added)	0.963 (0.954-0.972)	<0.0001
History of diabetes	1.131 (0.929-1.376)	0.2
History of malignancy	0.793 (0.677-0.929)	0.004
Diagnostic group : cardiovascular	0.936 (0.738-1.186)	0.6
Diagnostic group : respiratory	0.802 (0.660-0.975)	0.03
Diagnostic group : other	0.694 (0.571-0.844)	0.0003
Sepsis on admission	0.891 (0.778-1.021)	0.1
Randomization to IIT	1.198 (1.054-1.361)	0.006
Admitted to surgical ICU	1.029 (0.863-1.225)	0.8
Admission blood glucose (per mg/dl added)	0.999 (0.998-1.000)	0.03
Baseline CRP (per mg/ml added)	0.999 (0.999-1.000)	0.01

Hazard ratios and 95% confidence intervals are shown after correction for classical baseline characteristics and risk factors, as well as for the randomized intervention. A hazard ratio below 1

505 indicates a decreased likelihood of earlier discharge alive from the ICU or the hospital at any time.

506 Hence, higher sCD163 levels increase the risk of an extended stay in ICU or hospital.

507

FIGURE LEGENDS

Fig. 1 sCD163 levels in relation to mortality, bacteremia and organ dysfunction

a. Baseline sCD163 levels are shown for short- and long-stay patients who died in ICU, patients who acquired bacteremia, and patients who developed cholestatic liver dysfunction or new kidney injury versus those who did not. Data are expressed as box plots, where the central lines indicate the medians, the boxes the interquartile ranges and the whiskers the 10th and 90th percentiles. b. Time profiles of sCD163 are shown for short- and long-stay patients who died in ICU, patients who acquired bacteremia, and patients who developed cholestatic liver dysfunction or new kidney injury versus those who did not. They are presented as mean $\pm$ standard error of the change from baseline for last ICU day (LD) levels of short-stay patients and day 5, day 10, day 15 and last ICU day for long-stay patients. *P*-values were calculated by repeated measures ANOVA with baseline and last day levels for short-stay patients and with baseline, day 5 and last day levels for long-stay patients (as change from baseline). Numbers of the patients analyzed are shown below the graphs.

Fig. 2 ROC curves for prediction of mortality and cholestatic liver dysfunction with baseline sCD163 versus CRP

ROC curves are shown with black lines and the corresponding 95% CIs with grey lines. The optimal cut-offs for prediction by baseline sCD163 of mortality and liver dysfunction with corresponding sensitivity and specificity are sCD163 > 2.515 mg/L, 63.9%, 63.0% and sCD163 > 3.75 mg/L, 55.1%, 81.2%, respectively. For baseline CRP this is CRP > 102.1 mg/L, 64.7%, 49.4% and CRP > 129.7 mg/L, 52.0%, 55.0%, respectively.

Fig. 3 Impact of strict blood glucose control with insulin therapy during ICU stay on sCD163 levels

Data are shown at baseline and on the last day in ICU for short-stay patients and at baseline, day 5, day 15 and last day in ICU for long-stay patients. They are expressed as box plots, where the central

lines indicate the medians, the boxes the interquartile ranges and the whiskers the 10th and 90th percentiles.

Fig. 4 Impact of critical illness and intensive insulin therapy on hepatic gene expression of CD163

Relative mRNA levels of CD163 are shown as box plots, where the central lines indicate the medians, the boxes the interquartile ranges and the whiskers the 10th and 90th percentiles.

Figure 1

Figure 2

Figure 3

Figure 4