Elsevier Editorial System(tm) for Journal of Clinical and Experimental Hepatology
Manuscript Draft

Manuscript Number: JCEH-D-18-00121R1

Title: The macrophage activation marker soluble CD163 is associated with early allograft dysfunction following liver transplantation

Article Type: Original Article

Keywords: Liver transplantation; graft dysfunction; sCD163; macrophages

Corresponding Author: Dr. Karen Louise Thomsen,

Corresponding Author's Institution: Aarhus University Hospital

First Author: Karen Louise Thomsen

Order of Authors: Karen Louise Thomsen; Francis P Robertson; Peter Holland-Fischer; Brian R Davidson; Rajeshwar P Mookerjee; Holger J Møller; Rajiv Jalan; Henning Grønbæk

Abstract: Background / Objectives: Soluble (s)CD163, a macrophage activation marker, is up-regulated in conditions with macrophage proliferation and activation. Elevated sCD163 levels have been associated with liver disease severity and progression. During liver transplantation the implanted liver is exposed to ischemia and reperfusion injury resulting in an acute inflammatory response and macrophage activation. The relationship between sCD163 levels during liver transplantation and the development of early allograft dysfunction (EAD) has not been investigated.

Methods: We included 27 cirrhosis patients (age 55 (range 32-72) years, 23 men) on the waiting list for liver transplantation. Alcohol and viral hepatitis were the most frequent causes for cirrhosis. Patients were characterised by standard biochemistry and clinical disease severity scores. Information about donor, graft, and course of the liver transplantation was recorded. sCD163 levels were measured at time of liver transplant prior to surgery, 2 hours post reperfusion and then at 24 hours post transplantation.

Results: We observed above normal sCD163 levels at baseline (5.9 (4.7-8.8) mg/L). Two hours after reperfusion, sCD163 levels increased significantly from baseline (8.4 (7.4-10.9) mg/L; $P<0.01$). 24-hours after transplantation, sCD163 levels were significantly reduced compared to baseline (3.7 (2.9-5.5) mg/L; $P<0.01$). However, in patients with EAD (n=16), sCD163 levels were increased compared to patients without EAD (4.1 (3.2-7.4) vs. 3.1 (2.8-3.8) mg/L; $P=0.03$).

Conclusions: We observed elevated sCD163 levels in patients with EAD after liver transplantation confirming macrophage activation to play a role in EAD. Thus, sCD163 may be used as an early marker for EAD after liver transplantation.

The macrophage activation marker soluble CD163 is associated with early allograft dysfunction following liver transplantation

Karen Louise Thomsen[1,2], Francis P Robertson[3], Peter Holland-Fischer[2], Brian R Davidson[3], Rajeshwar P Mookerjee[2], Holger J Møller[4], Rajiv Jalan[2], Henning Grønbæk[1]

[1] Department of Hepatology & Gastroenterology, Aarhus University Hospital, Aarhus, Denmark

[2] Liver Failure Group, UCL Institute for Liver and Digestive Health, University College London, United Kingdom

[3] Division of Surgery and Interventional Science, Royal Free Campus, University College London, United Kingdom

[4] Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark

Correspondence:

Karen Louise Thomsen, MD, PhD

Department of Hepatology and Gastroenterology,

Aarhus University Hospital,

Palle Juul-Jensens Boulevard 99,

DK-8210 Aarhus N,

Denmark.

Phone (direct): +45 78453811

Fax: +45 78453897,

E-mail: karethom@rm.dk

Running title: Soluble CD163 in early allograft dysfunction

ABSTRACT

Background / Objectives: Soluble (s)CD163, a macrophage activation marker, is up-regulated in conditions with macrophage proliferation and activation. Elevated sCD163 levels have been associated with liver disease severity and progression. During liver transplantation the implanted liver is exposed to ischemia and reperfusion injury resulting in an acute inflammatory response and macrophage activation. The relationship between sCD163 levels during liver transplantation and the development of early allograft dysfunction (EAD) has not been investigated.

Methods: We included 27 cirrhosis patients (age 55 (range 32-72) years, 23 men) on the waiting list for liver transplantation. Alcohol and viral hepatitis were the most frequent causes for cirrhosis. Patients were characterised by standard biochemistry and clinical disease severity scores. Information about donor, graft, and course of the liver transplantation was recorded. sCD163 levels were measured at time of liver transplant prior to surgery, 2 hours post reperfusion and then at 24 hours post transplantation.

Results: We observed above normal sCD163 levels at baseline (5.9 (4.7-8.8) mg/L). Two hours after reperfusion, sCD163 levels increased significantly from baseline (8.4 (7.4-10.9) mg/L; P<0.01). 24-hours after transplantation, sCD163 levels were significantly reduced compared to baseline (3.7 (2.9-5.5) mg/L; P<0.01). However, in patients with EAD (n=16), sCD163 levels were increased compared to patients without EAD (4.1 (3.2-7.4) vs. 3.1 (2.8-3.8) mg/L; P=0.03).

Conclusions: We observed elevated sCD163 levels in patients with EAD after liver transplantation confirming macrophage activation to play a role in EAD. Thus, sCD163 may be used as an early marker for EAD after liver transplantation, but larger studies are warranted to validate these findings.

Word count: *248*

Key words*:* Liver transplantation, graft dysfunction, sCD163, macrophages

INTRODUCTION

CD163 is a scavenger receptor expressed exclusively on monocytes and macrophages.[1, 2] CD163 is shed into the circulation as soluble (s)CD163 and sCD163 levels increase during inflammation and macrophage activation.[3-5] More than 80% of body macrophages reside in the liver as so-called Kupffer cells and they are activated as part of the innate immune system in response to liver injury. We have previously demonstrated that sCD163 is associated with severity of various liver diseases from only slightly elevated levels in non-alcoholic fatty liver disease (NAFLD) [6] to very high sCD163 levels in patients with acute liver failure,[1] acute viral hepatitis [7] and alcoholic hepatitis.[8] Also in patients with liver cirrhosis, sCD163 levels are elevated [9, 10] and a prognostic marker for clinical decompensation and disease progression.[11-14]

Patients with end-stage cirrhotic liver disease have a poor prognosis unless they are offered liver transplantation. During transplantation the cirrhotic liver with activated macrophages is explanted and a new liver is inserted. The liver graft, however, is exposed to a hostile environment of ischemia during preservation, reperfusion injury and surgical stress during implantation resulting in inflammation and graft dysfunction, which may lead to fibrosis and decreased graft survival.[15, 16] Hepatic ischemia/reperfusion (I/R) injury is a multifactorial process involving various cell types and pro-inflammatory mediators.[17] Kupffer cells are responsible for the initial pro-inflammatory reaction during the early phase of reperfusion and their activation and formation of reactive oxygen species are considered pivotal mechanisms of I/R injury after liver transplantation.[18, 19] Early allograft dysfunction (EAD) is a clinical definition describing severe cases of I/R injury and associated with poor graft function and increased morbidity and mortality after liver transplantation.[20] Definitions of EAD vary but all are based on markers of hepatic function during the first week of transplantation. However, the standard liver function tests (e.g. transaminases, INR, bilirubin) measured to reflect graft function are all 'late events' in the evolution of liver injury. Since liver macrophage activation is predominant during the initial reperfusion period,[19] sCD163 levels may increase prior to the standard laboratory tests and be a potential marker for EAD.

The aim of the present study was to investigate whether the early events during and within the first 24 hours of liver transplantation is associated with Kupffer cell activation determined by sCD163 levels and whether levels correlate with the severity of graft dysfunction. We hypothesised that sCD163 increases following reperfusion, reflecting the severity of reperfusion injury and may predict EAD. We measured sCD163 concentrations during liver transplantation

at time 0 and 2 hours after reperfusion and again 24 hours after transplantation. For mechanistic linkage, inflammatory markers were also evaluated. Information regarding the liver donor and recipient and the course of the liver transplantation was recorded and for 7 days post-transplant daily standard liver biochemistry was measured for assessment of EAD.

MATERIAL AND METHODS

Subjects, study design and ethics

We included 27 cirrhosis patients admitted to the Royal Free London NHS Hospital Trust, UK for a liver transplant between 2014 and 2015. Patients were included if they were ≥18 years of age, had a clinical, radiological, or histological diagnosis of cirrhosis and were on the waiting list for liver transplantation. Exclusion criteria were acute or subacute liver failure, peripheral vascular disease, blood disorders, HIV infection and sepsis. Alcohol abstention was a prerequisite for being transplanted. Please refer to the original trial protocol for the exhaustive list.[21]

All patients were characterised by the clinical disease severity score Model for End-Stage Liver Disease (MELD) [22] and standard biochemistry at baseline and on day 1, day 3 and day 7. Information regarding donor, graft and course of the liver transplantation was recorded. sCD163 and various cytokine concentrations were measured in peripheral arterial blood collected during liver transplantation at baseline (following induction of anaesthesia but before abdominal incision), 2 hours post-reperfusion of the graft and again 24 hours post-operatively.

The study conformed to the Declaration of Helsinki, and written informed consent was obtained from all persons before participation. The protocol was approved by the NHS National Research Ethics Service (11/H0720/4) and the Royal Free Hospital/University College London medical school ethical committee (8191) and was registered in ClinicalTrials.gov (NCT00796588). The study was part of a protocol set up to investigate the effect of remote ischaemic preconditioning on outcomes of liver transplant and therefore some of the liver biochemistry and cytokine data have been published previously.[23] For the current study, further ethical approval was obtained to collect blood samples from patients and therefore only the remaining 27 patients were included unselectively. None of the sCD163 data described here have been published before. The remote ischaemic preconditioning (RIPC) had no effect on EAD (RIPC group: 10/16 (63%) vs. sham group: 6/11 (55%), p=0.68) or sCD163 levels 24 hours post-transplant (RIPC group: 3.7 (3.2-6.4) vs. sham group: 3.3 (2.9-5.4), p=0.41).

Liver transplantation

The grafts were identified and retrieved through the UK National Organ Retrieval Service according to national standards of organ retrieval from deceased donors. Following aortic cannulation, all grafts were perfused in situ with cold University of Wisconsin (UW) solution (Bridge to Life, Columbia, SC, USA)) at a maximum pressure of 200

mmHg. On removal the grafts were further flushed with ice-cold UW solution on the backbench via the hepatic artery, portal vein and the bile duct. The grafts were then sterile packaged in cold UW solution and transported to the recipient hospital on ice.

The recipients were monitored intraoperatively via arterial and central venous catheters. Implantation of the liver graft was performed by standard piggy-back and caval replacement techniques. Venovenous bypass was not employed in any patient in this study. Grafts were flushed with 500–1000 ml warm 4.5% human albumin solution (Bio Products Laboratory, Elstree, UK) via the portal vein immediately prior to blood re-perfusion to remove residual UW solution and waste material accumulated during cold ischaemia. One gram of methylprednisolone was given intravenously during the anhepatic phase as part of standard anaesthetic protocol.

Biochemical analyses

The plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, alkaline phosphatase (ALP), albumin, international normalized ratio (INR), prothrombin time (PT), creatinine, urea, hemoglobin (Hb) and C-reactive protein (CRP) concentrations and white blood cell (WBC) and platelet counts were measured immediately following collection by routine analytical methods.

Soluble CD163

Blood samples for the assessment of plasma sCD163 were centrifuged, separated and stored at -80°C until analysis. sCD163 was assessed using an in-house sandwich enzyme-linked immunosorbent assay (ELISA) as previously described.[24]

Cytokines

Blood samples for the assessment of plasma interleukin-6 (IL-6), tumor necrosis factor α (TNFα), IL-8, IL-10 and IL-17 were placed immediately on ice, centrifuged, separated and stored at -80°C until analysis. IL-6, TNFα and IL-10 were measured by LEGENDplex Human Th Cytokine Mix and Match Panel and IL-8 and IL-17 were measured by specific ELISA kits (BioLegend UK Ltd., London, UK, all).

Time-zero biopsies

In 22 (81%) of the transplantations, a liver biopsy was taken from the implanted liver 2 hours after reperfusion. Two biopsies were unsuitable for assessment leaving 20 (74%) patients with a histologically evaluation of the reperfused graft. The biopsies were reviewed by an experienced histopathologist at the Royal Free Hospital as part of routine clinical practice. Standard histological parameters were described including steatosis, preservation of portal tracts and liver architecture, any inflammation and suggestion of I/R injury.[25]

Statistics analysis

All statistical analyses were done using STATA statistical software package (StataCorp, Tx, USA). Variables were tested for a normal distribution using qq-plots and histograms. Variables showing skewed distributions were logarithmically transformed for further analysis. The changes in sCD163, cytokine levels and standard biochemistry were analysed by an analysis of variance with the measurement time used as the within-subjects factor and the patient ID used as the between-subjects factor. Differences in continuous variables between EAD and non-EAD patients were assessed using Student's t-test, whereas categorical variables were tested using Pearson χ^2 test. The relationships between the sCD163 concentrations and other variables were analysed by Spearman's rank correlation. Normally distributed continuous parameters are presented as mean ± SD, log-transformed data as median (IQR) and categorical variables as frequencies and percentages. P-values <0.05 were considered statistically significant.

RESULTS

Recipients and donor characteristics

We prospectively included 27 patients with liver cirrhosis (age 55 (range 32-72) years; 23 men (85%); BMI 27±5; MELD score 14±5) admitted for liver transplantation between 2014-2015. The aetiologies included viral (n=9), alcohol (n=8), viral plus alcohol (n=3), primary sclerosing cholangitis (n=4), non-alcoholic steatohepatitis (n=2), or autoimmune hepatitis (n=1), among these 9 also had hepatocellular carcinoma (HCC) within Milan criteria *(Table 1)*. The majority of liver grafts were from donors after brain death (DBD, 81%) (age 45 (range 14-69) years; BMI 26±5). The mean cold ischaemic time (CIT) was 509±145 min, the mean warm ischaemic time (WIT) was 88±23 min and 41% of the grafts had a degree of steatosis *(Table 1)*. The laboratory data for the recipients are provided in Table 2. AST, ALT, bilirubin and INR levels peaked immediately after transplant and were nearly back to normal on day 7.

Early allograft dysfunction

Sixteen patients (59%) were diagnosed as having EAD based on the following criteria: bilirubin ≥10 mg/dL on day 7, INR ≥1.6 on day 7 and/or AST or ALT >2000 IU/L within the first 7 days [26]. Recipients who developed EAD had increased body weight at baseline compared to non-EAD recipients (P<0.05). Also the BMI of the donors was higher in the EAD group (P<0.05) and in accordance with this, liver grafts with steatosis were more prevalent in the EAD group (56% vs. 18%; P<0.05) *(Table 1)*. As AST and ALT levels are used to define EAD, it was expected that their values were increased in the EAD group at all time points post-transplant except AST levels on day 7 compared to non-EAD recipients *(Table 2)*.

sCD163

The patients had increased sCD163 levels (5.9 (4.7-8.8) mg/L (median (IQR), normal range 0.69–3.86 mg/L [27]) at baseline. Two hours after reperfusion, sCD163 levels further increased to 8.4 (7.4-10.9) mg/L (P<0.01), whereas sCD163 levels 24 hours after transplantation were significantly reduced compared to baseline (3.7 (2.9-5.5) vs. 5.9 (4.7-8.8) mg/L; P<0.01). However, in patients who developed EAD, sCD163 levels 24 hours post-transplant were elevated compared to patients without EAD (4.1 (3.2-7.4) vs. 3.1 (2.8-3.8) mg/L; P=0.03), whereas this difference was not observed at baseline or 2 hours post-reperfusion *(Figure 1)*.

Cytokines

IL-6, IL-8, IL-10 and IL-17 levels all peaked 2 hours post-reperfusion and went back to normal levels 24 hours post-transplant. No significant differences in cytokine levels were observed between patients with and without EAD, however a tendency towards increased IL-10 levels in EAD patients was observed 2 hours post-reperfusion (680 (453-1297) vs. 420 (338-561) pg/mL P=0.07), *Table 3*. TNFα levels were lower 24 hours post-transplant compared to baseline, but again no differences were observed in EAD versus non-EAD patients.

sCD163 - correlations

Patients who received a steatotic liver graft had increased sCD163 levels 24 hours after transplantation compared to patients receiving a graft without steatosis (5.7 (4.1-7.4) vs. 3.2 (2.9-3.7) mg/L; P<0.001) and a positive correlation between elevated sCD163 levels and an increased degree of steatosis was observed (rho=0.64; P<0.001). Likewise, donor BMI correlated with sCD163 levels (rho=0.39; P<0.05). No correlations were found between duration of CIT or WIT and the degree of reperfusion injury in graft biopsies (time-zero biopsy) and sCD163 levels post-transplant.

sCD163 levels measured 24 hours post-transplantation correlated with ALT levels at the same time point (rho=0.41; P=0.04), with the highest ALT within the first 7 days (rho=0.39; P=0.05) and tended to correlate with ALT levels on day 3 (P=0.11). Also, the 24 hours post-transplant sCD163 levels strongly correlated with increased PT and INR levels measured on day 3 (rho=0.75; P<0.001, both) *(Figure 2)*.

sCD163 correlated with IL-10 levels when measured 2 hours post-reperfusion (rho=0.43; P=0.02) and 24 hours post-transplant (rho=0.46; P=0.02). Also IL-6 levels correlated with sCD163, 24 hours post-transplant (rho=0.46; P=0.02). No other correlations were observed between sCD163 and the cytokines measured.

DISCUSSION

Early graft dysfunction is a serious clinical condition post liver transplant associated with an increased morbidity and mortality. The central finding of this study was that 24 hours after liver transplantation, sCD163 levels were increased in patients who developed EAD, whereas sCD163 levels were close to the normal range in recipients without EAD. This suggest that macrophage activation is an early and key factor in the development and progression of EAD and that sCD163 levels may be used clinically as an early predictor for EAD after liver transplantation and help identifying which patients may benefit from more intensive medical support or even timely relisting.

The criteria for defining EAD varies among studies and it follows that the reported incidence of EAD varies depending on the criteria used.[15, 26, 28] In the present study, we used a definition, which has been successfully validated in a large multicentre study [26] and satisfactorily reflects overall graft function within the first week after transplantation. We found donor and recipient risk factors known to contribute to EAD development,[28-31] however, the EAD incidence in our cohort (59%) was remarkably high which might be explained by a high percentage of steatotic liver grafts in the EAD group compared to other studies [15, 28, 32]; and the use of deceased cardiac death (DCD) grafts in 25% of patients, which are well-known donor-related risk factors.[33, 34] Our data reflects the generalised trend in the increasing use of more steatotic and marginal grafts, necessitated by decreasing suitable donor availability and increasing demand. Also other studies have reported EAD rates that are higher than most commonly reported, [28, 35, 36] probably explained by the differences in donor populations. [37-39] Other known risk factors for EAD are long CIT, WIT and high donor age, which in the present study, were equally distributed between EAD and non-EAD patients. Notably, all our EAD patients were male, which is not known as a risk factor for EAD.

The patients were the remaining 27 patients in a randomised clinical trial enrolling 40 patients and so was an unselected group of patients. The trial aimed to examine whether RIPC could reduce I/R injury after liver transplantation. RIPC was found to be safe and feasible, but showed no evidence of clinical benefit.[23] In keeping with this, we found no differences in the rate of EAD or sCD163 levels between the intervention and the sham group. One third of our patients had HCC and as expected milder pre-transplant liver disease severity (data not shown) due to exception points being awarded resulting in earlier transplantation. However, no differences in rate of EAD development or sCD163 levels at baseline or 24 hours post-transplant were observed compared to non-HCC patients. Kanzankov et al. reported similar

sCD163 levels in patients with cirrhotic HCC and chronic liver disease (CLD) and so, sCD163 levels seem to be determined by the disease stage of CLD and not the burden of HCC.[40]

As expected, baseline sCD163 levels were higher in cirrhotic patients undergoing liver transplant than levels reported in healthy individuals, a finding which has been previously reported.[9, 10, 12, 14] Interestingly, the sCD163 levels were near normal in patients without EAD as early as 24 hours after liver transplantation. The mean cold ischemic time for the liver graft was as high as 9 hours in these non-EAD patients, and still the macrophages seemed to be less activated 24 hours post-operatively compared to the ones in the explanted liver, suggesting that macrophage activation is a transient event in those with an uncomplicated transplant. The plasma half-life of sCD163 is 12-24 hours after endotoxin administration in healthy man,[27] however in the setting of major surgery the plasma kinetics of sCD163 may be altered due to other factors such as infusion of fluids and blood products. Also the administration of corticosteroids per-operatively for transplantation may lead to increased sCD163 due to increased gene-expression.[41]

The rapid normalisation of sCD163 levels in patients with an uncomplicated recovery post-transplant in comparison to increased 24 h sCD163 levels in patients with EAD would suggest sCD163 as a potential biomarker of macrophage activation during reperfusion injury. However, the sample size in this study was too small for identifying a cut off of sCD163 levels to predict EAD. Kupffer cells are activated following graft reperfusion and particularly in patients with severe I/R injury.[18, 19] Therefore, it was not surprising that the sCD163 levels, 24h post-transplant correlated with ALT levels. However, sCD163 levels also strongly correlated with coagulation measures on day 3 indicating that coagulation tests disclose graft dysfunction at a much later stage compared to sCD163. Therefore, the data in this paper describing sCD163 as a potential biomarker for *early* diagnosis of EAD may also allow development of macrophage targeted therapies based on EAD's pathophysiology.

Our findings appear to contrast with a recent study in living donor liver transplantation in which low numbers of CD163 positive macrophages in donor liver biopsies were associated with poor graft function and adverse outcome.[42] However, the biopsies were obtained from the donor liver prior to hepatectomy and therefore, the CD163 expression do not evaluate the degree of macrophage activation in connection with I/R injury, but most likely reflect the 'quality' of the liver graft's resident macrophage population in a non-activated state. Unfortunately, pre-implantation biopsies were not available in our study.

Patients who received a steatotic liver graft were more prevalent in the EAD group in keeping with previous studies reporting a steatotic graft as an independent predictor of EAD.[28-30, 33, 43] These patients~~and~~ had higher sCD163 levels 24 hours after transplantation, which may suggest that sCD163 reflect the severity of liver steatosis as previously reported in NAFLD patients.[6, 44, 45] Amongst patients receiving a non-steatotic graft, sCD163 levels 24 h post-transplant whilst higher in the EAD group (median: 3.3 (3.1-3.7) vs. 2.9 (2.8-3.4)), were not significantly different from the non-EAD group likely due to our small sample size. This may suggest that hepatic macrophages are activated in liver steatosis, as reflected in increased sCD163 levels, and their activation may partly be involved in EAD development in recipients of steatotic grafts.

Most of the measured cytokines increased 2h post-reperfusion and were nearly back to normal 24h post-transplant. However, we observed no significant differences in concentrations between non-EAD and EAD patients, before, during or after transplant, which is in contrast to our sCD163 findings and in contrast to a previous study by Friedman et al.[46] However, we observed a correlation between sCD163 and IL-6 and IL-10 levels 24h post-transplant. Induction of NF-κB-associated genes in Kupffer cells is known to be an early event after I/R injury and through activation of this system, activated Kupffer cells secrete both pro- and anti-inflammatory mediators including IL-6 and IL-10. Activated Kupffer cells also increase the expression of CD163 receptors, which are cleaved and shed into the circulation as sCD163 after Toll-like receptor stimulation by inflammatory stimuli. We showed that sCD163, and not pro-inflammatory cytokines, was able to detect EAD early after transplantation. This might be explained by the fact that sCD163 is a specific marker of macrophage activation whereas cytokines are produced by a variety of immune cells and in liver transplantation, graft I/R outcome is more associated with activation of resident macrophages than the inflammatory cell infiltrates. Also, cytokines have shorter half-lives with more marked fluctuations in plasma levels compared to sCD163. Moreover, sCD163 is stable and remarkably resistant to sample processing in contrast to a number of cytokines and inflammatory markers.[24]

Several studies have investigated the effect of Kupffer cell depletion or inactivation on hepatic I/R injury in liver transplantation models using various agents.[47-50] Results are conflicting, but in several studies preventive effects have been demonstrated. Kupffer cell depletion induced by pre-treatment with gadolinium chloride attenuated graft reperfusion injury after transplantation in rats [50] and in pigs.[49] Also carbon monoxide ameliorates I/R injury through

down-regulation of Kupffer cell responses and in the same study gadolinium chloride again inhibited pro-inflammatory up-regulation of Kupffer cells.[48] These experimental data suggest macrophages play an important role in EAD. In man, other potential strategies to reduce reperfusion injury have been investigated. In a randomized study in patients undergoing liver resection, preoperative methylprednisolone administration reduced aminotransferases, bilirubin and inflammatory cytokines as well as postoperative complications.[51] In the current liver transplant study, 1 gram of methylprednisolone was given intravenously during the anhepatic phase as part of the standard anaesthetic per operative protocol and all patients continued on 16 mg/day of methylprednisolone after transplantation as part of their immunosuppressive medication to prevent graft rejection. This treatment dampens the immune response to I/R injury post-transplant and could thereby reduce hepatic damage and the risk of EAD.

In conclusion, we observed elevated levels of the macrophage activation marker sCD163 in patients with EAD early after liver transplantation, which suggest macrophage activation to play a role in EAD. We suggest that sCD163 may be used as an early marker for EAD after liver transplantation, but larger studies are warranted to validate these findings.

ACKNOWLEDGEMENTS

This study was generously supported by grants from the Medical Research Foundation for Central Denmark Region, Novo Nordisk Foundation, the Research Council at Aarhus University Hospital and Savværksejer Jeppe Juhl og hustru Ovita Juhls Mindelegat.

Conflicts of interest

Holger J Møller is an inventor for the CD163-dexamethasone conjugate and a minority shareholder in Affinicon Aps. Rajiv Jalan has on-going research collaboration with Yaqrit and Takeda. He is also inventor for a drug, L-ornithine phenyl acetate (OCR-002) which UCL has licensed to Ocera Therapeutics. He is also the founder of UCL spin-out company Yaqrit ltd. and Cyberliver ltd. Henning Grønbæk has obtained funding from Intercept and Abbvie. All other authors have nothing to disclose.

REFERENCES

1. Moller H J, Gronbaek H, Schiodt F V, Holland-Fischer P, Schilsky M, Munoz S, et al. Soluble CD163 from activated macrophages predicts mortality in acute liver failure. *J Hepatol* 2007; 47(5): 671-6.
2. Kristiansen M, Graversen J H, Jacobsen C, Sonne O, Hoffman H J, Law S K, et al. Identification of the haemoglobin scavenger receptor. *Nature* 2001; 409(6817): 198-201.
3. Weaver L K, Hintz-Goldstein K A, Pioli P A, Wardwell K, Qureshi N, Vogel S N, et al. Pivotal advance: activation of cell surface Toll-like receptors causes shedding of the hemoglobin scavenger receptor CD163. *J Leukoc Biol* 2006; 80(1): 26-35.
4. Hintz K A, Rassias A J, Wardwell K, Moss M L, Morganelli P M, Pioli P A, et al. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. *J Leukoc Biol* 2002; 72(4): 711-7.
5. Etzerodt A, Maniecki M B, Moller K, Moller H J, Moestrup S K. Tumor necrosis factor {alpha}-converting enzyme (TACE/ADAM17) mediates ectodomain shedding of the scavenger receptor CD163. *J Leukoc Biol* 2010.
6. Kazankov K, Barrera F, Moller H J, Rosso C, Bugianesi E, David E, et al. The macrophage activation marker sCD163 is associated with morphological disease stages in patients with non-alcoholic fatty liver disease. *Liver Int* 2016; 36(10): 1549-57.
7. Hiraoka A, Horiike N, Akbar S M, Michitaka K, Matsuyama T, Onji M. Expression of CD163 in the liver of patients with viral hepatitis. *Pathol Res Pract* 2005; 201(5): 379-84.
8. Sandahl T D, Gronbaek H, Moller H J, Stoy S, Thomsen K L, Dige A K, et al. Hepatic Macrophage Activation and the LPS Pathway in Patients With Alcoholic Hepatitis: A Prospective Cohort Study. *Am J Gastroenterol* 2014.
9. Holland-Fischer P, Gronbaek H, Sandahl T D, Moestrup S K, Riggio O, Ridola L, et al. Kupffer cells are activated in cirrhotic portal hypertension and not normalised by TIPS. *Gut* 2011; 60(10): 1389-93.
10. Gronbaek H, Sandahl T D, Mortensen C, Vilstrup H, Moller H J, Moller S. Soluble CD163, a marker of Kupffer cell activation, is related to portal hypertension in patients with liver cirrhosis. *Aliment Pharmacol Ther* 2012; 36(2): 173-80.
11. Rode A, Nicoll A, Moller H J, Lim L, Angus P W, Kronborg I, et al. Hepatic macrophage activation predicts clinical decompensation in chronic liver disease. *Gut* 2013; 62(8): 1231-2.
12. Waidmann O, Brunner F, Herrmann E, Zeuzem S, Piiper A, Kronenberger B. Macrophage activation is a prognostic parameter for variceal bleeding and overall survival in patients with liver cirrhosis. *J Hepatol* 2013; 58(5): 956-61.
13. Tornai T, Vitalis Z, Sipeki N, Dinya T, Tornai D, Antal-Szalmas P, et al. Macrophage activation marker, soluble CD163, is an independent predictor of short-term mortality in patients with cirrhosis and bacterial infection. *Liver Int* 2016; 36(11): 1628-38.
14. Rainer F, Horvath A, Sandahl T D, Leber B, Schmerboeck B, Blesl A, et al. Soluble CD163 and soluble mannose receptor predict survival and decompensation in patients with liver cirrhosis, and correlate with gut permeability and bacterial translocation. *Aliment Pharmacol Ther* 2018; 47(5): 657-64.
15. Hudcova J, Scopa C, Rashid J, Waqas A, Ruthazer R, Schumann R. Effect of early allograft dysfunction on outcomes following liver transplantation. *Clin Transplant* 2017; 31(2).
16. Deschenes M, Belle S H, Krom R A, Zetterman R K, Lake J R. Early allograft dysfunction after liver transplantation: a definition and predictors of outcome. National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. *Transplantation* 1998; 66(3): 302-10.

17. Olthoff K M. Molecular pathways of regeneration and repair after liver transplantation. *World J Surg* 2002; 26(7): 831-7.
18. Teoh N C, Farrell G C. Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection. *J Gastroenterol Hepatol* 2003; 18(8): 891-902.
19. Jaeschke H, Farhood A. Neutrophil and Kupffer cell-induced oxidant stress and ischemia-reperfusion injury in rat liver. *Am J Physiol* 1991; 260(3 Pt 1): G355-62.
20. Deschenes M. Early allograft dysfunction: causes, recognition, and management. *Liver Transpl* 2013; 19 Suppl 2: S6-8.
21. Robertson F P, Goswami R, Wright G P, Fuller B, Davidson B R. Protocol for a prospective randomized controlled trial of recipient remote ischaemic preconditioning in orthotopic liver transplantation (RIPCOLT trial). *Transplantation research* 2016; 5: 4.
22. Kamath P S, Wiesner R H, Malinchoc M, Kremers W, Therneau T M, Kosberg C L, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; 33(2): 464-70.
23. Robertson F P, Goswami R, Wright G P, Imber C, Sharma D, Malago M, et al. Remote ischaemic preconditioning in orthotopic liver transplantation (RIPCOLT trial): a pilot randomized controlled feasibility study. *HPB : the official journal of the International Hepato Pancreato Biliary Association* 2017; 19(9): 757-67.
24. Moller H J, Hald K, Moestrup S K. Characterization of an enzyme-linked immunosorbent assay for soluble CD163. *Scand J Clin Lab Invest* 2002; 62(4): 293-9.
25. Datta Gupta S, Hudson M, Burroughs A K, Morris R, Rolles K, Amlot P, et al. Grading of cellular rejection after orthotopic liver transplantation. *Hepatology* 1995; 21(1): 46-57.
26. Olthoff K M, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* 2010; 16(8): 943-9.
27. Moller H J. Soluble CD163. *Scand J Clin Lab Invest* 2012; 72(1): 1-13.
28. Hoyer D P, Paul A, Gallinat A, Molmenti E P, Reinhardt R, Minor T, et al. Donor information based prediction of early allograft dysfunction and outcome in liver transplantation. *Liver Int* 2015; 35(1): 156-63.
29. Marsman W A, Wiesner R H, Rodriguez L, Batts K P, Porayko M K, Hay J E, et al. Use of fatty donor liver is associated with diminished early patient and graft survival. *Transplantation* 1996; 62(9): 1246-51.
30. Lee D D, Croome K P, Shalev J A, Musto K R, Sharma M, Keaveny A P, et al. Early allograft dysfunction after liver transplantation: an intermediate outcome measure for targeted improvements. *Ann Hepatol* 2016; 15(1): 53-60.
31. Pulitano C, Joseph D, Sandroussi C, Verran D, Ho P, Debiasio A, et al. Postreperfusion microcirculatory derangements after liver transplantation: Relationship to hemodynamics, serum mediators, and outcome. *Liver Transpl* 2017; 23(4): 527-36.
32. Verran D, Kusyk T, Painter D, Fisher J, Koorey D, Strasser S, et al. Clinical experience gained from the use of 120 steatotic donor livers for orthotopic liver transplantation. *Liver Transpl* 2003; 9(5): 500-5.
33. Strasberg S M, Howard T K, Molmenti E P, Hertl M. Selecting the donor liver: risk factors for poor function after orthotopic liver transplantation. *Hepatology* 1994; 20(4 Pt 1): 829-38.
34. Lee D D, Singh A, Burns J M, Perry D K, Nguyen J H, Taner C B. Early allograft dysfunction in liver transplantation with donation after cardiac death donors results in inferior survival. *Liver Transpl* 2014; 20(12): 1447-53.

35. Salvalaggio P R, Felga G E, Afonso R C, Ferraz-Neto B H. Early allograft dysfunction and liver transplant outcomes: a single center retrospective study. *Transplant Proc* 2012; 44(8): 2449-51.
36. Selten J W, Verhoeven C J, Heedfeld V, Roest H P, De Jonge J, Pirenne J, et al. The release of microRNA-122 during liver preservation is associated with early allograft dysfunction and graft survival after transplantation. *Liver Transpl* 2017; 23(7): 946-56.
37. Schlitt H J, Loss M, Scherer M N, Becker T, Jauch K W, Nashan B, et al. [Current developments in liver transplantation in Germany: MELD-based organ allocation and incentives for transplant centres]. *Z Gastroenterol* 2011; 49(1): 30-8.
38. Savier E, Dondero F, Vibert E, Eyraud D, Brisson H, Riou B, et al. First experience of liver transplantation with type 2 donation after cardiac death in France. *Liver Transpl* 2015; 21(5): 631-43.
39. Nemes B, Gaman G, Polak W G, Gelley F, Hara T, Ono S, et al. Extended-criteria donors in liver transplantation Part II: reviewing the impact of extended-criteria donors on the complications and outcomes of liver transplantation. *Expert review of gastroenterology & hepatology* 2016; 10(7): 841-59.
40. Kazankov K, Rode A, Simonsen K, Villadsen G E, Nicoll A, Moller H J, et al. Macrophage activation marker soluble CD163 may predict disease progression in hepatocellular carcinoma. *Scand J Clin Lab Invest* 2016; 76(1): 64-73.
41. Goldstein J I, Goldstein K A, Wardwell K, Fahrner S L, Goonan K E, Cheney M D, et al. Increase in plasma and surface CD163 levels in patients undergoing coronary artery bypass graft surgery. *Atherosclerosis* 2003; 170(2): 325-32.
42. Nigam N, Bihari C, Lal D, Rastogi A, Kumar S, Pamecha V, et al. Donor CD163 and nestin-positive cells predict graft function in living donor liver transplant. *Clin Transplant* 2018; 32(3): e13197.
43. Ploeg R J, D'alessandro A M, Knechtle S J, Stegall M D, Pirsch J D, Hoffmann R M, et al. Risk factors for primary dysfunction after liver transplantation--a multivariate analysis. *Transplantation* 1993; 55(4): 807-13.
44. Mueller J L, Feeney E R, Zheng H, Misdraji J, Kruger A J, Alatrakchi N, et al. Circulating Soluble CD163 is Associated with Steatohepatitis and Advanced Fibrosis in Nonalcoholic Fatty Liver Disease. *Clinical and translational gastroenterology* 2015; 6: e114.
45. Kazankov K, Tordjman J, Moller H J, Vilstrup H, Poitou C, Bedossa P, et al. Macrophage activation marker soluble CD163 and non-alcoholic fatty liver disease in morbidly obese patients undergoing bariatric surgery. *J Gastroenterol Hepatol* 2015; 30(8): 1293-300.
46. Friedman B H, Wolf J H, Wang L, Putt M E, Shaked A, Christie J D, et al. Serum cytokine profiles associated with early allograft dysfunction in patients undergoing liver transplantation. *Liver Transpl* 2012; 18(2): 166-76.
47. Schemmer P, Bradford B U, Rose M L, Bunzendahl H, Raleigh J A, Lemasters J J, et al. Intravenous glycine improves survival in rat liver transplantation. *Am J Physiol* 1999; 276(4 Pt 1): G924-32.
48. Tomiyama K, Ikeda A, Ueki S, Nakao A, Stolz D B, Koike Y, et al. Inhibition of Kupffer cell-mediated early proinflammatory response with carbon monoxide in transplant-induced hepatic ischemia/reperfusion injury in rats. *Hepatology* 2008; 48(5): 1608-20.
49. Von Frankenberg M, Golling M, Mehrabi A, Nentwich H, Klar E, Kraus T W. Donor pretreatment with gadolinium chloride improves early graft function and survival after porcine liver transplantation. *Transpl Int* 2003; 16(11): 806-13.

50. Zhu H, Marco C, Gianfranco F. Early changes of graft function, cytokines and superoxide dismutase serum levels after donor liver denervation and Kupffer cell depletion in a rat-to-rat liver transplantation model. *Hepatobiliary Pancreat Dis Int* 2009; 8(2): 152-6.

51. Aldrighetti L, Pulitano C, Arru M, Finazzi R, Catena M, Soldini L, et al. Impact of preoperative steroids administration on ischemia-reperfusion injury and systemic responses in liver surgery: a prospective randomized study. *Liver Transpl* 2006; 12(6): 941-9.

Table 1. Baseline characteristics of recipients and donors.

		Early Allograft Dysfunction		
	All patients	**No (n=11)**	**Yes (n=16)**	**P value**
Recipient characteristics				
Age (years)	55 ± 9	53 ± 10	57 ± 9	P=0.36
Sex m/f (%)	85/15	67/36	100/0	P<0.01
Weight (kg)	81 ± 17	73 ± 13	86 ± 18	P=0.043
BMI	27 ± 5	25 ± 4	28 ± 5	P=0.20
MELD	14 ± 5	13 ± 4	15 ± 6	P=0.46
Hepatocellular carcinoma, n (%)	9 (33)	5 (45)	4 (25)	P=0.27
Remote ischaemic preconditioning, n (%)	16 (59)	6 (55)	10 (63)	P=0.68
Red cells during transplant (range)	2 (0-4)	2 (0-3)	4 (2-5)	P=0.49
Kidney failure, n (%)	8 (30)	3 (27)	5 (31)	P=0.82
Lenght of time in ITU (days)	3 (2-4)	3 (2-6)	3 (2-4)	P=0.92
Lenght of time in hospital (days)	17 (10-25)	16 (10-26)	18 (12-21)	P=0.76
Donor characteristics				
Age (years)	45 ± 17	42 ± 20	47 ± 15	P=0.47
BMI	26 ± 5	24 ± 4	27 ± 5	P=0.049
Type of donor				P=0.30
- **deceased brain death,** n (%)	22 (81)	10 (91)	12 (75)	
- **deceased cardiac death,** n (%)	5 (19)	1 (9)	4 (25)	
Lenght of time in ITU (days)	2 (2-4)	2 (2-4)	3 (2-5)	P=0.70
Cold Ischaemic Time (min)	509 ± 145	526 ± 183	497 ± 118	P=0.62
Warm Ischaemic Time (min)	44 ± 13	41 ± 16	46 ± 11	P=0.34
Graft, steaosis, n (%)	11 (41)	2 (18)	9 (56)	P=0.048
- mild (<30%)	9 (33)	2 (18)	7 (44)	
- moderate (30-60%)	2 (7)	0 (0)	2 (13)	

Baseline characteristics of recipients and donors for all patients and divided into recipients with no early allograft dysfunction (EAD) (No) and recipients who developed EAD (Yes).

Normally distributed data are presented as mean ± SD
Log-transformed data are presented as median (IQR)

Table 2. Standard biochemistry.

	Baseline Early Allograft Dysfunct.		Day 1 post-transplant Early Allograft Dysfunct.		Day 3 post-transplant Early Allograft Dysfunct.		Day 7 post-transplant Early Allograft Dysfunct.	
	No (n=11)	**Yes (n=16)**	**No (n=11)**	**Yes (n=16)**	**No (n=11)**	**Yes (n=16)**	**No (n=11)**	**Yes (n=16)**
AST (U/L)	92 (58-125)	68 (52-81)	423 (244-712)	2366 ** (1369-3620)	104 (91-163)	418 # (260-660)	54 (33-92)	54 (49-60)
ALT (U/L)	65 (54-68)	38 (30-62)	446 (332-672)	1679 ** (1187-2848)	271 (199-306)	979 ** (481-1701)	107 (77-174)	250 # (167-418)
Bilirubin (μmol/L)	24 (18-63)	47 (32-77)	42 (16-72)	64 (40-86)	24 (10-53)	43 (27-63)	28 (21-36)	36 (17-54)
ALP (U/L)	151 (101-438)	120 (93-186)	54 (46-156)	58 (45-74)	108 (71-193)	103 (70-158)	193 (155-299)	240 (168-348)
Albumin (g/L)	33.5 ±7.5	33.4 ±4.4	25.7 ±5.9	26.5 ±5.2	25.2 ±3.8	26.9 ±3.8	24.7 ±3.9	27.5 ±4.2
INR	1.4 (1.1-1.6)	1.4 (1.2-1.6)	1.7 (1.4-1.8)	1.8 (1.5-2.4)	1.1 (1.0-1.2)	1.2 (1.0-1.3)	1.0 (0.9-1.2)	1.0 (0.9-1.1)
PT	15 (13-19)	16 (13-18)	20 (16-21)	20 (17-27)	13 (12-14)	13 (12-14)	11 (10-13)	11 (11-12)
Sodium (mmol/L)	139 ±4	136 * ±5	142 ±3	138 * ±4	137 ±4	137 ±3	135 ±3	136 ±3
Potassium (mmol/L)	4.4 ±0.5	4.3 ±0.5	5.0 ±0.6	5.0 ±0.5	4.8 ±0.5	4.7 ±0.5	4.2 ±0.4	4.3 ±0.4
Creatinine (μmol/L)	85 ±19	84 ±30	109 ±57	116 ±48	114 ±63	107 ±73	76 ±32	68 ±21
Urea	6.0	6.3	8.7	10.6	12.3	12.8	8.0	6.9

(mmol/L)	±2.1	±2.7	±3.2	±3.6	±6.0	±5.7	±5.4	±2.4
CRP (mg/L)	7 (4-16)	8 (3-13)	64 (50-101)	68 (54-73)	31 (10-39)	31 (23-47)	26 (14-47)	34 (23-61)
WBC ($x10^9$/L)	4.8 ±1.5	6.2 ±3.0	11.2 ±3.8	12.9 ±7.4	10.0 ±4.9	10.4 ±6.5	12.2 ±3.9	10.7 ±6.6
Hb (g/dL)	11.3 ±2.5	10.7 ±1.5	9.3 ±1.1	9.3 ±1.4	8.8 ±1.6	8.7 ±1.3	9.4 ±1.7	8.9 ±1.6
Platelets ($x10^9$/L)	77 (41-108)	82 (71-180)	72 (37-107)	62 (39-101)	65 (41-94)	47 (33-98)	79 (63-182)	100 (71-184)

Standard biochemistry at baseline and on day 1, 3 and 7 in patients with no early allograft dysfunction (EAD) (No) and in patients who developed EAD (Yes).

* P<0.05 compared to no early allograft dysfunction
** P<0.001 compared to no early allograft dysfunction
# P=0.001 compared to no early allograft dysfunction
Normally distributed data are presented as mean ± SD
Log-transformed data are presented as median (IQR)

Table 3. Plasma cytokine levels.

	Baseline pre-transplant	2 h post-reperfusion	24 h post-transplant	Comparing all 3 time points	Baseline vs. 2 h post-rep.	Baseline vs. 24 h post-tx	2 h post-rep. vs. 24 h post-tx
IL-6	14 (8-50)	644 (317-1132)	22 (10-43)	P<0.0001	P<0.0001	P=0.83	P<0.0001
TNFα	8.0 (3.5-71.5)	6.9 (3.5-37.0)	5.5 (3.5-8.6)	P<0.0001	P=0.20	P=0.02	P=0.09
IL-8	0.9 (0.0-3.3)	29.1 (14.8-52.1)	0.9 (0.0-3.1)	P<0.0001	P<0.0001	P=0.73	P<0.0001
IL-10	4.2 (3.7-8.4)	561 (345-854)	7.4 (4.6-35.3)	P<0.0001	P<0.0001	P=0.26	P<0.0001
IL-17	2.2 (1.7-3.1)	2.9 (1.8-9.1)	1.9 (0.8-2.3)	P<0.0001	P=0.02	P=0.32	P=0.002

Plasma cytokine levels (pg/mL) before liver transplantation (baseline), 2 h post-reperfusion of the liver graft and 24 h post-operatively in all patients.

Data are presented as median (IQR)

FIGURE LEGENDS

Figure 1. sCD163 levels before, during and after liver transplantation.

Plasma sCD163 levels before liver transplantation (baseline), 2 h post-reperfusion of the liver graft and 24 h post-operatively in patients with no early allograft dysfunction (EAD) (n=11) and patients with EAD (n=16). The solid horizontal lines indicate the median values, the boxes the IQR and the error bars 95th percentiles. * P<0.03 compared to no EAD.

Figure 2. Relationship between sCD163 and ALT (A) and INR (B) levels.

Relationship between sCD163 and alanine aminotransferase (ALT) levels 24 h post-operatively (rho=0.41; P=0.04) (A) and international normalized ratio (INR) 3 days post-transplant (rho=0.75; P<0.001) (B) in patients with EAD (black dots) and no EAD (white dots). The linear regression line shows the correlation.

Figure 1

Click here to download high resolution image

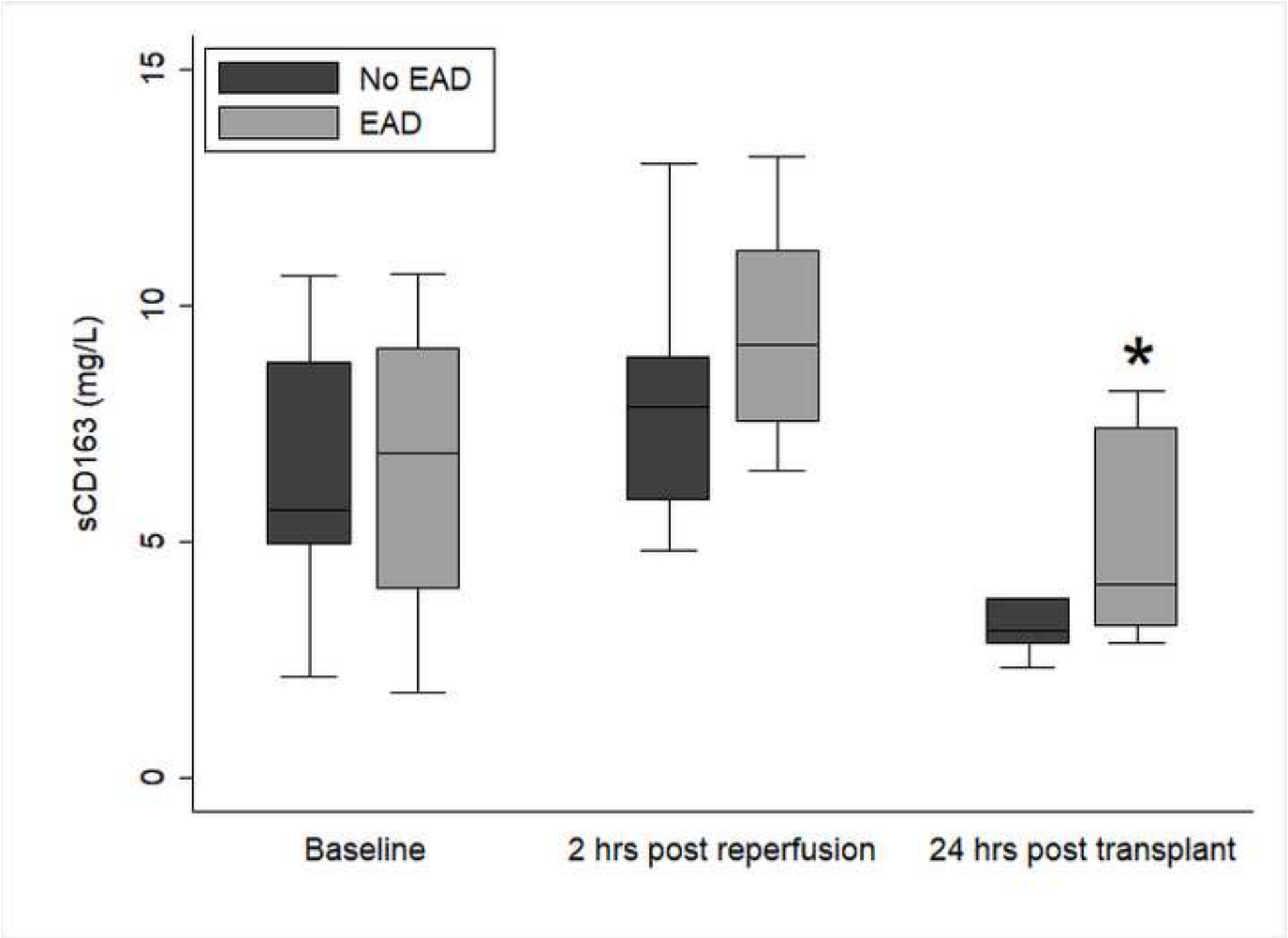

Figure 2A

Click here to download high resolution image

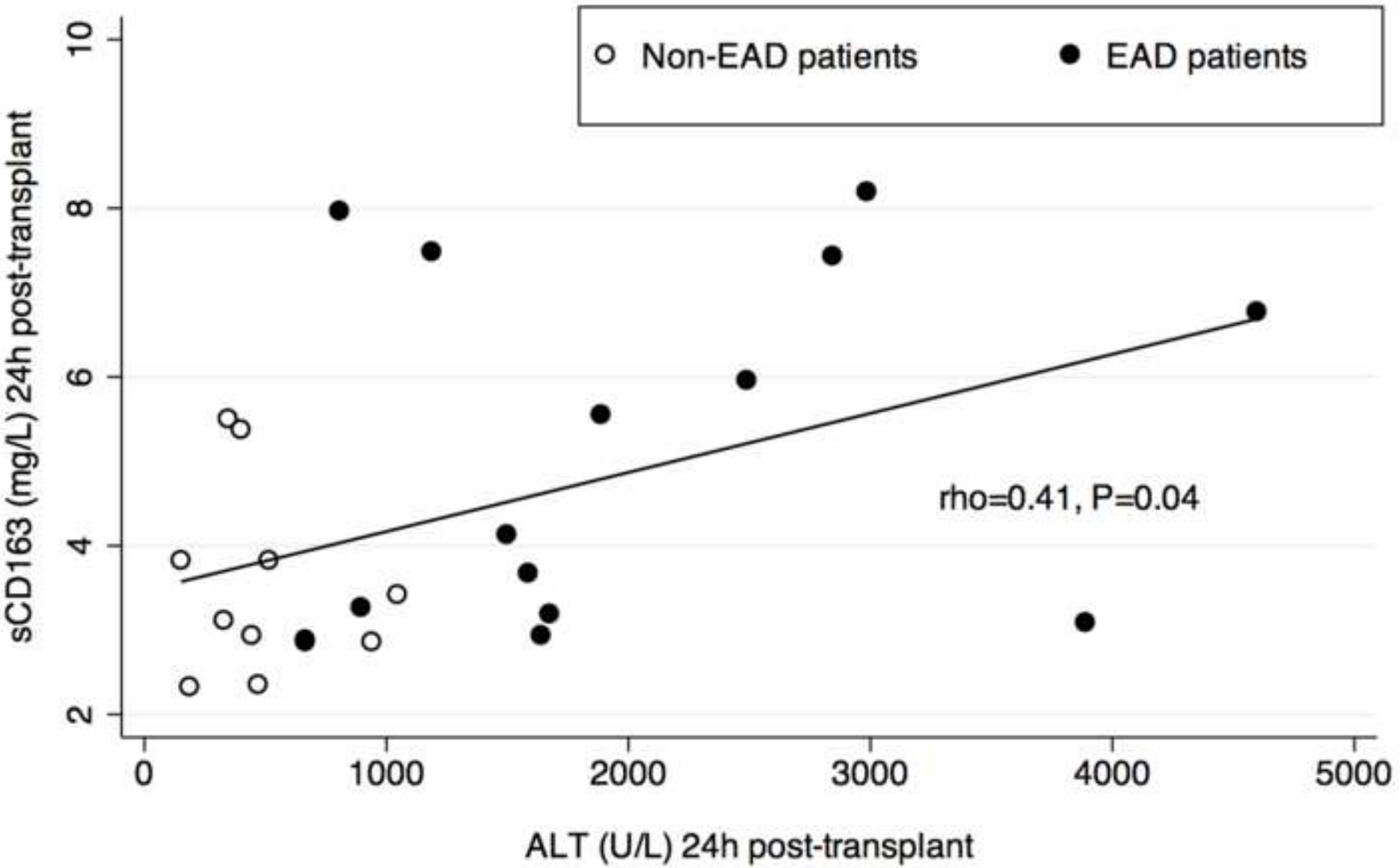

Figure 2B

Click here to download high resolution image