The Macrophage Activation Marker Soluble CD163 is Associated With Early Allograft Dysfunction After Liver Transplantation

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Background/Objectives: Soluble CD163 (sCD163), a macrophage activation marker, is upregulated in conditions of 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 ischaemia 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 consumption and viral hepatitis were the most frequent causes for cirrhosis. Patients were characterised by standard biochemical analysis and based on clinical disease severity scores. Information about donor, graft and course of the liver transplantation was recorded. sCD163 levels were measured at the time of liver transplantation before surgery, 2 h after reperfusion, and then at 24 h after transplantation. **Results:** We observed above-normal sCD163 levels at baseline (5.9 mg/L [4.7–8.8]). Two hours after reperfusion, sCD163 levels increased significantly from baseline (8.4 mg/L [7.4–10.9]; $P < 0.01$). Twenty-four hours after transplantation, sCD163 levels were significantly reduced compared with baseline (3.7 mg/L [2.9–5.5]; $P < 0.01$). However, in patients with EAD ($n = 16$), sCD163 levels were increased compared with 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. (J CLIN EXP HEPATOL 2019;9:302–311)

CD163 is a scavenger receptor expressed exclusively on monocytes and macrophages.^{1,2} CD163 is shed into the circulation as soluble CD163 (sCD163), and sCD163 levels increase during

inflammation and macrophage activation.^{3–5} More than 80% of body macrophages reside in the liver in the form of 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 nonalcoholic fatty liver disease (NAFLD)⁶ to very high sCD163 levels in patients with acute liver failure,¹ acute viral hepatitis⁷ and alcoholic hepatitis.⁸ Also, in patients with liver cirrhosis, sCD163 levels are elevated^{9,10}, 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 implanted. The liver graft, however, is exposed to a hostile environment of ischaemia 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

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Abbreviations: ALK: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CIT: cold ischaemic time; CRP: C-reactive protein; DBD: deceased brain death; DCD: deceased cardiac death; EAD: Early allograft dysfunction; ELISA: enzyme-linked immunosorbent assay; Hb: haemoglobin; IL: interleukin; INR: international normalised ratio; IQR: interquartile range; I/R: ischaemia/reperfusion; MELD: Model for End-Stage Liver Disease; NAFLD: nonalcoholic fatty liver disease; NF- κ B: nuclear factor- κ B; PT: prothrombin time; sCD163: soluble CD163; TNF- α : tumour necrosis factor α ; WBC: white blood cell; WIT: warm ischaemic time

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ischaemia/reperfusion (I/R) injury is a multifactorial process involving various cell types and proinflammatory mediators.¹⁷ Kupffer cells are responsible for the initial proinflammatory 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, international normalised ratio [INR] and bilirubin) measured to reflect graft function are all 'late events' in the evolution of liver injury. Because liver macrophage activation is predominant during the initial reperfusion period,¹⁹ sCD163 levels may increase before 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 h of liver transplantation are associated with Kupffer cell activation determined by sCD163 levels and whether levels correlate with the severity of graft dysfunction. We hypothesised that sCD163 increases after reperfusion, reflecting the severity of reperfusion injury and may predict EAD. We measured sCD163 concentrations during liver transplantation at time 0 and 2 h after reperfusion and again 24 h 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 after transplantation, standard liver biochemistry was measured daily 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 transplantation between 2014 and 2015. Patients were included if they aged ≥ 18 years; 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 abstinence 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 biochemical analysis at baseline and on day 1, day 3 and day 7. Information regarding the 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 (after induction of anaesthesia but before abdominal incision), 2 h after reperfusion of the graft and again 24 h postoperatively.

The study conformed to the Declaration of Helsinki, and written informed consent was obtained from all patients 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](https://www.clinicaltrials.gov) (NCT00796588). The study was part of a protocol set up to investigate the effect of remote ischaemic preconditioning (RIPC) on outcomes of liver transplantation, and therefore, some of the liver biochemistry and cytokine data have been published previously.²³ For the present 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 RIPC had no effect on EAD (RIPC group, 10/16 [63%] vs. sham group, 6/11 [55%]; $P = 0.68$) or sCD163 levels 24 h after transplantation (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. After 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 on ice to the recipient hospital.

The recipients were monitored intraoperatively using arterial and central venous catheters. Implantation of the liver graft was performed by standard piggy-back and caval replacement techniques. Venovenous bypass was not used in any patient in this study. Grafts were flushed with 500–1000 ml of warm 4.5% human albumin solution (Bio Products Laboratory, Elstree, UK) via the portal vein immediately before blood reperfusion 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, INR, prothrombin time (PT), creatinine, urea, haemoglobin (Hb) and C-reactive protein (CRP) concentrations and white blood cell (WBC) and platelet counts were measured immediately after 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 were placed immediately on ice, centrifuged, separated and stored at -80°C until analysis for the assessment of plasma interleukin-6 (IL-6), tumour necrosis factor α (TNF α), IL-8, IL-10 and IL-17. IL-6, TNF α and IL-10 levels were measured by LEGENDplex Human Th Cytokine Mix and Match Panel, and IL-8 and IL-17 levels were measured using 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 two hours after reperfusion. Two biopsies were unsuitable for assessment, leaving 20 (74%) patients with a histological 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, including steatosis, preservation of portal tracts and liver architecture, any inflammation and suggestion of I/R injury, were described.²⁵

Statistics Analysis

All statistical analyses were performed 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 $\pm$ SD, log-transformed data as median (interquartile range [IQR]) and categorical variables as fre-

quencies 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%]; body mass index [BMI] 27 ± 5 ; MELD score 14 ± 5) admitted for liver transplantation between 2014 and 2015. The aetiologies included viral ($n = 9$), alcohol ($n = 8$), viral plus alcohol ($n = 3$), primary sclerosing cholangitis ($n = 4$), nonalcoholic steatohepatitis ($n = 2$) or autoimmune hepatitis ($n = 1$); among these patients, 9 also had hepatocellular carcinoma (HCC) within Milan criteria (Table 1). Most 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 transplantation 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 with 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 after transplantation, except AST levels on day 7, compared with non-EAD recipients (Table 2).

Soluble CD163

The patients had increased sCD163 levels (median [IQR], 5.9 [4.7–8.8] mg/L; normal range, 0.69–3.86 mg/L²⁷) 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 h after transplantation significantly reduced compared with 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 h after transplantation were elevated compared with 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 h after reperfusion (Figure 1).

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
Length of time in ITU (days)	3 (2–4)	3 (2–6)	3 (2–4)	P = 0.92
Length 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)	
Length 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, steatosis, 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).

BMI, body mass index; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; SD, standard deviation.

Cytokines

IL-6, IL-8, IL-10 and IL-17 levels all peaked 2 h after reperfusion and went back to normal levels 24 h after transplantation. 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 h after reperfusion (680 [453–1297] vs. 420 [338–561] pg/mL $P = 0.07$), Table 3. TNF α levels were lower at 24 h after transplant than at 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 h after transplantation compared with 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 ($\rho = 0.64$; $P < 0.001$). Similarly, 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 after transplant.

sCD163 levels measured 24 h after 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-h posttransplant 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 h after reperfusion ($\rho = 0.43$; $P = 0.02$) and 24 h after

Table 2 Standard Biochemistry.

	Baseline		Day 1 posttransplant		Day 3 posttransplant		Day 7 posttransplant	
	Early allograft dysfunction		Early allograft dysfunction		Early allograft dysfunction		Early allograft dysfunction	
	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 (mmol/L)	6.0 ± 2.1	6.3 ± 2.7	8.7 ± 3.2	10.6 ± 3.6	12.3 ± 6.0	12.8 ± 5.7	8.0 ± 5.4	6.9 ± 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).

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; Hb, haemoglobin; INR, internationalised ratio; IQR, interquartile range; PT, prothrombin time; SD, standard deviation; WBC, white blood cell.

Figure 1 sCD163 levels before, during and after liver transplantation. Plasma sCD163 levels before liver transplantation (baseline), 2 h after reperfusion of the liver graft and 24 h postoperatively 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. IQR, interquartile range; sCD163, soluble CD163.

transplantation ($\rho = 0.46$; $P = 0.02$). Also, IL-6 levels correlated with sCD163 24 h after transplantation ($\rho = 0.46$; $P = 0.02$). No other correlations were observed between sCD163 and the cytokines measured.

DISCUSSION

Early graft dysfunction is a serious clinical condition after liver transplantation associated with an increased morbidity and mortality. The central finding of this study was that 24 h 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 suggests 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 vary 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 that has been successfully validated in a large multicentre study²⁶ 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 with other studies^{15,28,32} and the use of deceased cardiac death (DCD) grafts in 25% of patients, which are well-known

Table 3 Plasma Cytokine Levels.

	Baseline pretransplant	2 h after reperfusion	24 h after transplantation	Comparing all 3 time points	Baseline vs. 2 h after reperfusion	Baseline vs. 24 h after transplantation	2 h after reperfusion vs. 24 h after transplantation
IL-6	14 (8–50)	644 (317–1132)	22 (10–43)	$P < 0.0001$	$P < 0.0001$	$P = 0.83$	$P < 0.0001$
TNF α	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 postoperatively in all patients. Data are presented as median (IQR).

IL, interleukin; IQR, interquartile range; TNF α , tumour necrosis factor α .

Figure 2 Relationship between sCD163 and ALT (A) and INR (B) levels. Relationship between sCD163 and alanine aminotransferase (ALT) levels 24 h postoperatively ($\rho = 0.41$; $P = 0.04$) (A) and international normalised ratio (INR) 3 days after 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. EAD, early allograft dysfunction; sCD163, soluble CD163.

donor-related risk factors.^{33,34} Our data reflect 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 and 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 un-

selected 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 pretransplant liver disease severity (data not shown) due to exception points being awarded, resulting in earlier transplantation. However, no differences in the rate of EAD development or sCD163 levels at baseline or 24 h after transplantation were observed compared with 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 transplantation than levels reported in healthy individuals, a finding that has been previously reported.^{9,10,12,14} Interestingly, the sCD163 levels were near normal in patients without EAD as early as 24 h after liver transplantation. The mean CIT for the liver graft was as high as 9 h in these non-EAD patients, and still the macrophages seemed to be less activated 24 h postoperatively than 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 h 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 peroperatively 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 after transplantation 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 after graft reperfusion and particularly in patients with severe I/R injury.^{18,19} Therefore, it was not surprising that the sCD163 levels, 24 h after transplantation, 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 than sCD163. Therefore, the data in this article describing sCD163 as a potential biomarker for early diagnosis of EAD may also allow development of macrophage-targeted therapies based on EAD pathophysiology.

Our findings appear to contrast with a recent study on living donor liver transplantation in which low numbers of CD163-positive macrophages in donor liver biopsies were associated with poor graft function and adverse outcomes.⁴² However, the biopsies were obtained from the donor liver before 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 nonactivated state. Unfortunately, preimplantation 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 had higher sCD163 levels 24 h after transplantation, which may

suggest that sCD163 reflect the severity of liver steatosis as previously reported in NAFLD patients.^{6,44,45} Among patients receiving a nonsteatotic graft, sCD163 levels 24 h after transplantation, although higher in the EAD group (median: 3.3 [3.1–3.7] vs. 2.9 [2.8–3.4]), were not significantly different from those in 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 2 h after reperfusion and were nearly back to normal 24 h after transplantation. 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 24 h after transplantation. Induction of nuclear factor- κ B-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 proinflammatory and antiinflammatory 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 proinflammatory 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 than 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 pretreatment with gadolinium chloride attenuated graft reperfusion injury after transplantation in rats⁵⁰ and pigs.⁴⁹ Also, carbon monoxide ameliorates I/R injury through downregulation of Kupffer cell responses, and in the same study, gadolinium chloride again inhibited proinflammatory upregulation of Kupffer cells.⁴⁸ These experimental data suggest that macrophages play an important role in EAD. In man, other potential strategies to reduce reperfusion injury have been investigated. In a randomised study on patients undergoing liver resection, preoperative methylprednisolone administration reduced

aminotransferases, bilirubin and inflammatory cytokines as well as postoperative complications.⁵¹ In the present liver transplant study, 1 g of methylprednisolone was given intravenously during the anhepatic phase as part of the standard anaesthetic peroperative 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 after transplantation 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.

CONFLICTS OF INTEREST

The authors have none to declare.

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