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Original Article

Increased macrophage activation marker soluble CD163 is associated with graft dysfunction and metabolic derangements in renal transplant recipients

Hayam El Aggan [a,*], *Sabah Mahmoud* [b], *Heba El Shair* [a], *Hazem Elabd* [a]

[a] *Department of Internal Medicine (Nephrology and Transplantation Unit), University of Alexandria, Alexandria, Egypt*

[b] *Department of Medical Biochemistry, University of Alexandria, Alexandria, Egypt*

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ABSTRACT

Background: Renal allograft is vulnerable to numerous insults and is associated with metabolic derangements. Macrophages are regulators of inflammation and play a role in obesity, lipid metabolism and insulin resistance (IR). The present study was designed to assess macrophage activation, reflected by serum soluble CD163 (sCD163), in renal transplant recipients (RTR) and its relation to chronic allograft dysfunction (CAD) and metabolic derangements.

Methods: Fifty recipients of renal transplantation (RT) [22 with stable renal function and 28 with CAD] and 20 age- and sex-matched healthy controls were enrolled in the study. Serum sCD163 and high sensitivity C-reactive protein (hsCRP) were measured using enzyme-linked immunosorbent assay. Anthropometric measurements, renal function, lipid profile and homeostatic model assessment of IR (HOMA-IR) were estimated. Renal interstitial fibrosis (IF) was graded in renal biopsies of CAD.

Results: RTR mean age was 38.84 ± 9.28 years and 83% of them were males. Post-transplant dyslipidemia, diabetes and IR (HOMA-IR >2) were present in 42%, 24% and 86% of RTR respectively. Serum sCD163 levels were significantly higher in RTR with stable renal function and CAD than in healthy controls (814.41 ± 59.62 ng/ml and 1021.21 ± 120.82 ng/ml vs. 602.90 ± 114.98 ng/ml respectively) and in RTR with CAD than in patients with stable renal function ($p < 0.001$). Serum sCD163 levels were positively correlated with body mass index, waist-to-hip ratio, worsening renal function, dyslipidemia, HOMA-IR and serum hsCRP in RTR and with the degree of renal IF in RTR with CAD ($p < 0.05$). ROC curve showed that serum sCD163 was superior to serum hsCRP in detecting CAD after RT (AUC = 0.972 vs. 0.753 respectively, $p = 0.001$).

Conclusion: Macrophage activation, reflected by increased circulating sCD163, may play a role in the development of CAD and metabolic derangements after RT. Serum sCD163 could be a potential biomarker for renal allograft dysfunction.

* *Corresponding author*. Department of Internal Medicine (Nephrology and Transplantation Unit), University of Alexandria, Elkhartoom Square, Elazaritta, Alexandria 21131, Egypt.
E-mail address: hayamelaggan@yahoo.com (H. El Aggan).

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At a glance of commentary

Scientific background on the subject

Renal allograft is vulnerable to numerous insults and is associated with metabolic derangements. Macrophages are regulators of inflammation and play a role in obesity, lipid metabolism and insulin resistance. Increased soluble CD163, a macrophage activation marker, has been found in acute and chronic inflammation, fibrotic diseases and metabolic disorders.

What this study adds to the field

Macrophage activation, reflected by increased circulating soluble CD163, may play a role in the development of chronic allograft dysfunction and metabolic derangements after renal transplantation. Serum soluble CD163 could be a potential biomarker for detecting renal allograft dysfunction and this has to be validated in clinical trials with large-scale population.

Renal transplantation (RT) is the preferred treatment modality of end-stage renal disease. Despite improving immunosuppressive protocols in RT, chronic allograft dysfunction (CAD) remains a major impediment to long-term graft survival [1]. CAD is characterized by a gradual worsening of renal function and histological changes including interstitial fibrosis/tubular atrophy (IF/TA), microvascular rarefaction and glomerulosclerosis [2]. The renal allograft is vulnerable to numerous injurious immune and non-immune insults. As a consequence, an innate immune response is triggered and inflammatory cells are recruited within the allograft [3] and contribute to progressive renal fibrosis and the pathogenesis of CAD [4]. Metabolic complications including weight gain, dyslipidemia and insulin resistance (IR) that are common following RT, are associated with chronic low-grade inflammation and may threaten graft function. These risk factors are often exacerbated by immunosuppressive drugs and are related to increased cardiovascular risk [5]. Controlling the activation of innate immunity/inflammatory responses could be a promising strategy to increase the graft survival and control post-transplant metabolic derangements [6].

Macrophages are key components of the innate immune system, which play important roles in the regulation of the inflammatory process and the maintenance of tissue homeostasis [7]. Two main macrophage phenotypes have been recognized; M1 and M2 macrophages. The M1 phenotype is proinflammatory, develops after exposure to microbial products such as lipopolysaccharide and interferon-γ (classical activation) and is characterized by secretion of proinflammatory cytokines (tumor necrosis factor-α (TNF-α), interferon-γ, interleukin (IL)-12 and IL-1β), microbicidal capacity and production of nitric oxide [8]. The M2 phenotype is induced by IL-4 and IL-13, transforming growth factor-β (TGF-β) and glucocorticoids (alternative activation) and in turn produces large amounts of IL-10, arginase-1 and TGF-β with surface expression of scavenger receptors. It has anti-inflammatory and immunoregulatory functions and is a key player in tissue remodeling, angiogenesis and fibrogenesis [9]. Macrophages demonstrate high plasticity and can be functionally polarized into M1 or M2 phenotype in response to local environmental cues [8]. A growing number of studies reported an important role of macrophages in acute and chronic kidney diseases [10,11] including damaged kidney allograft [12–14] and found that polarization of macrophages into M2 phenotype is involved in the pathogenesis of renal injury and fibrosis [11]. Moreover, macrophages may play a role in metabolic disorders including obesity, lipid metabolism, IR and diabetes mellitus [15,16].

Cluster of differentiation 163 (CD163) is a surface scavenger receptor for hemoglobin-haptoglobin complexes that is expressed exclusively on macrophages and monocytes. The receptor is a type-I transmembrane protein with a short cytoplasmic tail, a single transmembrane segment, and a large ectodomain composed of 9 extracellular consecutive scavenger receptor cysteine-rich domains type B [17]. CD163 exhibits strong anti-inflammatory properties and is upregulated in conditions with macrophage activation representing a switch to alternatively-activated (M2) phenotype in inflammation [18]. CD163 has both a membrane-bound variant and a soluble variant, which is present in the plasma and other tissue fluids. Soluble CD163 (sCD163) has been proposed to be a product of shedding of CD163 by proteolytic cleavage of the ectodomain upon macrophage activation in response to proinflammatory stimuli, oxidative stress and immune complexes cross-linking Fcγ receptors [17]. At least two enzymes have been implicated in this process: matrix metaloproteinase-9 and the inflammation regulated a disintegrin and metalloproteinase 17/TNF-α–cleaving enzyme (ADAM17/TACE) [19]. Increased plasma concentration of sCD163 has been found in conditions related to macrophage activation including acute and chronic inflammation [20], fibrotic diseases [21] and metabolic disorders [22]. However, there is a paucity of data on sCD163 in renal transplantation and its role in allograft dysfunction and post-transplant metabolic complications.

Therefore, the present work was designed to assess macrophage activation, reflected by serum soluble CD163 (sCD163), in renal transplant recipients (RTR) and its relation to chronic allograft dysfunction (CAD) and metabolic derangements.

Materials and methods

Study population

The present study included 50 recipients of RT for more than 6 months [22 RTR with stable renal function (serum creatinine ≤2 mg/dl) and 28 RTR with CAD (serum creatinine >2 mg/dl)], who were referred to the Nephrology and Transplantation Unit, Department of Internal Medicine, Main Alexandria University Hospital, Faculty of Medicine, Alexandria, Egypt. They were 41 males and 9 females, and their ages ranged between 20 and 57 years (mean ± SD = 38.84 ± 9.28 years). The patients were selected from 123 RTR after exclusion of viral infections, underlying chronic liver disease, pre-transplant diabetes mellitus or hyperlipidemia, connective tissue and

autoimmune diseases, other infections or inflammatory disorders, any kind of malignancy, cardiac and respiratory diseases and previous drug intake other than the immunosuppressive drugs. Also, 20 age- and sex-matched healthy subjects were included as a control group. They were 16 males and 4 females and their ages ranged between 23 and 55 years (mean ± SD = 38.25 ± 8.46 years) (Fig. 1). The study was approved by the Research Ethics Committee/the Institutional Review Board of the Faculty of Medicine, University of Alexandria and was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. Informed consent was obtained from all subjects included in the study.

All RTR were evaluated clinically as regards age, gender, original renal disorders, previous RT, number of attacks of acute rejection, other post-transplantation complications, viral infections [hepatitis C virus, hepatitis B virus, human immunodeficiency virus and cytomegalovirus] and immunosuppressive regimens [corticosteroids, cyclosporine and mycophenolate mofetil]. Therapeutic immunosuppressive drugs were monitored for all transplanted patients. Laboratory investigations included complete blood picture, complete urine analysis, renal function tests [blood urea nitrogen, serum creatinine, estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration formula [23] and urinary albumin/creatinine ratio (ACR)], lipid profile [serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG)], fasting plasma glucose (FPG) and fasting serum insulin (FSI) levels using a commercially-available enzyme immunoassay kit (DRG International, Inc. USA). Serum levels of high sensitivity C-reactive protein (hsCRP), a marker of systemic inflammation, were measured using enzyme-linked immunosorbent assay (ELISA) kit (Cusabio, Wuhan, Hubei Province, China).

Anthropometric measurements

Anthropometric measurements were performed including body mass index (BMI) and waist-to-hip ratio (WHR). BMI was calculated as weight (kg) divided by height (m^2) and was classified as underweight: <18.5 kg/m^2, normal: 18.5–24.9 kg/m^2, overweight: 25–29.9 kg/m^2 and obese: ≥30 kg/m^2. Weight and height were measured, with the subject standing, to the nearest 0.1 kg and 1 cm, respectively. WHR, a measure for central obesity, was measured as waist circumference (cm) divided by hip circumference (cm). Waist and hip circumferences were measured to the nearest 0.1 cm. The cut-off points for defining central obesity; WHR ≥0.90 in men and ≥0.85 in women [24].

Estimation of homeostasis model assessment of insulin resistance (HOMA-IR) index

Estimation of IR was performed using the homeostasis model assessment of IR (HOMA-IR) index calculated according to the

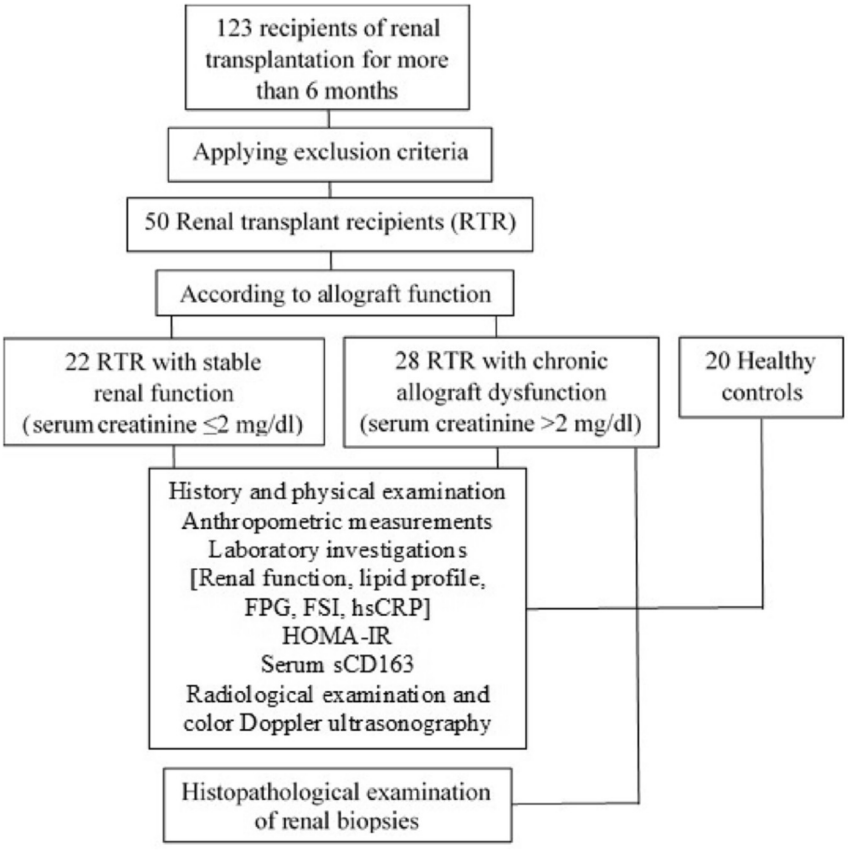

Fig. 1 Study design. Abbreviations used: FPG: Fasting plasma glucose; FSI: Fasting serum insulin; hsCRP: High sensitivity C-reactive protein; HOMA-IR: Homeostasis model assessment of insulin resistance; sCD163: Soluble CD163.

following equation: *FSI (μU/mL) × FBG (mg/dL)/405*. Insulin resistance was defined as the values of HOMA-IR exceeding 2.0 [25].

Measurement of soluble CD163 levels in serum by enzyme-linked immunosorbent assay

Quantitative determination of serum levels of sCD163 was performed using a commercially available standard sandwich ELISA kit (Boster Biological Technology, CA, USA) according to the manufacturer's instruction. Briefly, 0.1 ml of properly diluted serum samples and human CD163 standard solutions were added to empty wells of 96-well plate pre-coated with anti-human CD163 antibody and incubated at 37 °C for 90 min. A biotinylated anti-human CD163 antibody was added subsequently into each well and the plate was incubated at 37 °C for 60 min followed by washing with phosphate-buffered saline (PBS). Avidin-Biotin-Peroxidase Complex (ABC) was added and unbound conjugates were washed away with PBS or TBS buffer. Colorimetric detections were performed with tetramethylbenzidine (TMB) color developing agent and reactions were read at 450 nm. The relative optical density 450 was calculated as (the OD 450 of each well − the OD 450 of Zero well). The standard curve was plotted and the human sCD163 concentration of the samples was interpolated from the standard curve. For diluted serum samples, the dilution factor was multiplied to the concentrations from interpolation to obtain the concentration before dilution. The sensitivity of the kit was 0.15 ng/ml.

Radiological examination and color Doppler ultrasonography

Radiological examination of the transplanted kidneys was done for the detection of signs of acute rejection and the presence of surgical complications. Color Doppler ultrasonography was performed for assessment of renal and intrarenal vessels and renal resistive index (RRI) was calculated. An RRI value is > 0.70 is considered an indicator of increased renal vascular resistance [26].

Histopathological examination

Renal allograft biopsies obtained from RTR with CAD were fixed in 10% formalin solution, embedded in paraffin, sectioned (5 μm-thick) and subsequently stained with hematoxylin-eosin and trichrome stains were used to quantify histologic findings of CAD and the degree of renal interstitial fibrosis (IF) according to the Banff interstitial fibrosis (*ci*) score as follows: (1) *ci*0: Interstitial fibrosis in up to 5% of cortical area, (2) *ci*1: Interstitial fibrosis in 6–25% of the cortical area (mild interstitial fibrosis), (3) *ci*2: Interstitial fibrosis in 26–50% of the cortical area (moderate interstitial fibrosis), (4) *ci*3: Interstitial fibrosis in >50% of the cortical area (severe interstitial fibrosis) [27].

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Continuous data were presented as mean ± standard deviation (SD) and categorical data are presented as numbers and percentages. The normality of the data was determined by using Kolmogorov–Smirnov and Shapiro–Wilk tests. For normally-distributed continuous data, comparison between groups was done using the Student's *t*-test and One-way ANOVA test with *post hoc* test (Tukey) for pairwise comparisons. Kruskal–Wallis test was used for non-normally distributed data and when the test was positive, Dunn's for multiple comparisons test was done for pairwise comparisons in a *post hoc* fashion. For categorical data, Fisher's Exact test with Monte Carlo corrected significance was used for comparison between groups. Correlations between variables were analyzed using Spearman's rank test. Stratified analysis was performed using the Mantel-Haenszel formula to assess whether the relationship between serum sCD163 levels and renal allograft outcome was due to the confounding effect of other variables. The whole study population was divided into strata for each variable. Crude (unstratified) odds ratio (OR) of the whole patient group, stratum-specific OR, adjusted OR and 95% confidence intervals (CIs) were calculated. The receiver operating characteristic (ROC) curve was used to determine the sensitivity, specificity, cut-off value and area under the curve (AUC) with a 95% confidence interval of serum sCD163 and serum hsCRP in discriminating RTR with CAD from those with stable renal function. Positive predictive value (PPV) and negative predictive value (NPV) were calculated at the same cut-off values. The comparison between the two ROC curves was performed using MedCalc version 19.3 (MedCalc software Ltd., Ostend, Belgium). Statistical significance was assessed at $p < 0.05$. All calculated p values were two-tailed.

Results

Characteristics of subjects

The main characteristics of RTR with stable renal function and CAD and healthy controls are presented in Table 1. No significant differences in age and gender were observed between RTR and healthy controls ($p = 0.946$ and $p = 0.734$ respectively) and in the duration of transplantation between RTR with stable renal function and those with CAD ($p = 0.912$). Blood urea nitrogen, serum creatinine levels and urinary ACR were significantly higher in RTR with CAD than in those with stable renal function and healthy controls without statistically significant differences between RTR with stable renal function and healthy controls ($p < 0.001$ for all). Total leukocyte count, serum hsCRP levels, and RRI were significantly higher in RTR with stable renal function and CAD than in healthy controls and RTR with CAD than in those with stable renal function ($p < 0.001$ for all). By contrast, hemoglobin concentration and eGFR showed significant decreases in RTR with stable renal function and CAD compared with healthy controls and in RTR with CAD compared with those with stable renal function ($p < 0.001$ both). The degree of renal IF in RTR with CAD was mild (*ci*1) in 11 patients (39.3%), moderate (*ci*2) in 9 patients (32.1%) and severe (*ci*3) in 8 patients (28.6%).

Anthropometric measurements, lipid profile and insulin resistance in renal transplant recipients

Table 2 showed the metabolic parameters in RTR and healthy controls. BMI was significantly higher in RTR with stable renal function and CAD than in healthy controls with no statistically significant differences between RTR groups ($p < 0.001$). Waist-to-hip ratio, serum levels of TC, LDL-C and TG, FPG, FSI and HOMA-IR were significantly higher in RTR with stable renal function and CAD than in healthy controls and in RTR with CAD than in those with stable renal function ($p < 0.001$ for all). RTR with stable renal function and CAD showed a significant decrease in serum HDL-C levels compared with healthy controls with no statistically significant differences between RTR groups ($p = 0.003$). The frequency of post-transplant dyslipidemia and IR (HOMA-IR >2) were significantly higher in RTR with CAD than in RTR with stable renal function ($p < 0.001$ and $p = 0.002$ respectively) while there was no statistically significant difference in the frequency of post-transplant diabetes mellitus between RTR groups ($p = 0.853$).

Serum soluble CD163 levels were elevated in renal transplant recipients and were related to the development of chronic allograft dysfunction

Serum sCD163 levels ranged between 630 and 890 ng/ml in RTR with stable renal function, between 845 and 1245 ng/ml in RTR with CAD and between 420 and 750 ng/ml in healthy controls. Serum sCD163 levels were significantly higher in RTR with stable renal function and RTR with CAD than in healthy controls (814.41 ± 59.62 ng/ml and 1021.21 ± 120.82 ng/ml vs. 602.90 ± 114.98 ng/ml respectively) and in RTR with CAD than in those with stable renal function ($H = 57.313$, $p < 0.001$) (Fig. 2).

Elevated serum soluble CD163 levels were associated with progression of renal disease in renal transplant recipients

Spearman's analysis showed that serum sCD163 levels in RTR with stable renal function and RTR with CAD were positively correlated with serum creatinine ($r = 0.643$, $p = 0.001$ and $r = 0.420$, $p = 0.026$ respectively), urinary ACR ($r = 0.588$, $p = 0.004$ and $r = 0.452$, $p = 0.016$ respectively) and RRI ($r = 0.485$, $p = 0.022$ and $r = 0.623$, $p < 0.001$ respectively). In RTR with CAD, serum sCD163 were positively correlated with the degree of renal IF ($r = 0.827$, $p < 0.001$) and inversely correlated with eGFR ($r = -0.419$, $p = 0.026$) (Table 3).

Serum soluble CD163 levels were correlated with serum high sensitivity C-reactive protein levels and metabolic derangements in renal transplant recipients

Serum sCD163 levels in RTR with stable renal function and RTR with CAD were positively correlated with serum hsCRP levels ($r = 0.574$, $p = 0.005$ and $r = 0.697$, $p < 0.001$ respectively), BMI ($r = 0.500$, $p = 0.018$ and $r = 0.520$, $p = 0.005$ respectively), WHR ($r = 0.427$, $p = 0.047$ and $r = 0.504$, $p = 0.006$ respectively), serum levels of TC ($r = 0.540$, $p = 0.009$ and $r = 0.565$, $p = 0.002$ respectively), LDL-C ($r = 0.549$, $p = 0.008$ and $r = 0.575$, $p = 0.001$ respectively), and TG ($r = 0.619$, $p = 0.002$ and $r = 0.554$, $p = 0.002$ respectively), FSI ($r = 0.568$, $p = 0.006$ and $r = 0.766$,

Table 1 Characteristics of renal transplant recipients with stable renal function and chronic allograft dysfunction (CAD) and healthy controls.

Variables	Renal transplant recipients		Healthy controls (n = 20)	*p*-value[c]
	Stable renal function (n = 22)	CAD (n = 28)		
Age (years)	38.50 ± 11.13	39.11 ± 7.74	38.25 ± 8.46	0.946
Gender				
Male, *n* (%)	17 (77.3)	24 (85.7)	16 (80.0)	0.734[a]
Female, *n* (%)	5 (22.7)	4 (14.3)	4 (20.0)	
Renal transplantation duration (years)	7.23 ± 4.86	7.11 ± 2.69	–	0.912[b]
Hemoglobin (g/dl)	11.17 ± 1.74[d]	9.41 ± 1.16[d,e]	13.61 ± 1.13	<0.001
Total leukocyte count ($x10^3/mm^3$)	7.62 ± 1.22[d]	9.33 ± 1.29[d,e]	6.37 ± 1.40	<0.001
BUN (mg/dl)	40.82 ± 12.82	89.14 ± 28.26[d,e]	29.00 ± 5.51	<0.001
Creatinine (mg/dl)	1.11 ± 0.28	4.21 ± 1.39[d,e]	0.83 ± 0.11	<0.001
eGFR ($ml/min/1.73m^2$)	80.29 ± 21.93[d]	18.79 ± 7.95[d,e]	106.64 ± 10.28	<0.001
Urinary ACR (mg/g)	212.50 ± 102.77	1814.43 ± 996.72[d,e]	17.95 ± 6.78	<0.001
hsCRP (mg/l)	4.94 ± 3.54[d]	8.11 ± 3.79[d,e]	2.15 ± 1.09	<0.001
Renal resistive index	0.61 ± 0.05[d]	0.73 ± 0.07[d,e]	0.59 ± 0.05	<0.001
Renal interstitial fibrosis				
Mild, *n* (%)	–	11 (39.3)	–	
Moderate, *n* (%)	–	9 (32.1)	–	
Severe, *n* (%)	–	8 (28.6)	–	

Abbreviations: BUN: Blood urea nitrogen; eGFR: Estimated glomerular filtration rate; ACR: Albumin/creatinine ratio; hsCRP: High sensitivity C-reactive protein.

Continuous data are represented as mean ± SD and categorical data are represented as number and percentages.

[a] Fisher's Exact test with Monte Carlo corrected significance.

[b] Student's *t*-test.

[c] One-way ANOVA test with *post hoc* test (Tukey) for pairwise comparisons.

[d] Significant difference from healthy controls ($p < 0.05$).

[e] Significant difference from renal transplant recipients with stable renal function ($p < 0.05$).

Table 2 Anthropometric measurements, lipid profile and insulin resistance in renal transplant recipients with stable renal function and chronic allograft dysfunction (CAD) and healthy controls.

Variables	Renal transplant recipients		Healthy controls (n = 20)	p-value[b]
	Stable renal function (n = 22)	CAD (n = 28)		
Body mass index (kg/m^2)	24.64 ± 2.70[c]	27.59 ± 1.18[c]	21.55 ± 1.50	<0.001
Waist-to-hip ratio	0.90 ± 0.06[c]	1.07 ± 0.12[c,d]	0.78 ± 0.05	<0.001
Total cholesterol (mg/dl)	144.68 ± 38.02[c]	220.29 ± 42.16[c,d]	108.05 ± 12.54	<0.001
HDL-C (mg/dl)	39.82 ± 3.92[c]	37.21 ± 4.40[c]	42.90 ± 3.88	0.003
LDL-C (mg/dl)	114.95 ± 15.56[c]	153.36 ± 22.73[c,d]	82.90 ± 7.41	<0.001
Triglycerides (mg/dl)	173.73 ± 39.66[c]	203.00 ± 25.94[c,d]	98.20 ± 10.13	<0.001
Post-transplant dyslipidemia, *n* (%)	3 (13.6)	18 (64.3)	–	<0.001[a]
Fasting plasma glucose (mg/dl)	113.91 ± 15.15[c]	121.68 ± 8.41[c,d]	86.85 ± 6.90	<0.001
Fasting serum insulin (μIU/ml)	14.34 ± 6.22[c]	21.81 ± 9.86[c,d]	7.02 ± 2.08	<0.001
HOMA-IR	3.55 ± 1.45[c]	5.90 ± 2.73[c,d]	1.36 ± 0.43	<0.001
Insulin resistance, *n* (%)	15 (68.2)	28 (100.0)		0.002
Diabetes mellitus, *n* (%)	5 (22.7)	7 (25.0)	–	0.853[a]

Abbreviations: HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance.
Continuous data are represented as mean ± SD and categorical data are represented as number and percentages.
[a] Fisher's Exact test with Monte Carlo corrected significance.
[b] One-way ANOVA test with *post hoc* test (Tukey) for pairwise comparisons.
[c] Significant difference from healthy controls ($p < 0.05$).
[d] Significant difference from renal transplant recipients with stable renal function ($p < 0.05$).

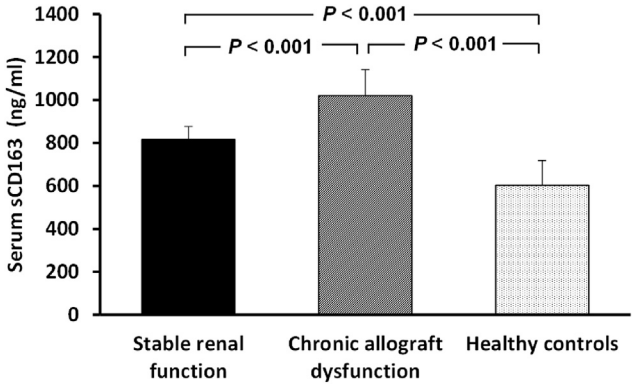

Fig. 2 Serum soluble CD163 (sCD163) levels (ng/ml) in renal transplant recipients (RTR) with stable renal function, RTR with chronic allograft dysfunction and healthy controls. Data are expressed as mean ± standard deviation [814.41 ± 59.62 ng/ml, 1021.21 ± 120.82 and 602.90 ± 114.98 ng/ml respectively, H = 57.313, $p < 0.001$, Kruskal–Wallis test with *post hoc* test (Dunn's for multiple comparisons test) for pairwise comparisons].

$p < 0.001$ respectively) and HOMA-IR (r = 0.587, $p = 0.004$ and r = 0.751, $p < 0.001$ respectively), and were inversely correlated with HDL-C levels (r = - 0.617, $p = 0.002$ and r = −0.579, $p = 0.001$ respectively) (Table 4).

Comparison between serum soluble CD163 and serum high sensitivity C-reactive protein as biomarkers for chronic allograft dysfunction after renal transplantation

The sensitivity, specificity, PPV and NPV of serum sCD163 in discriminating RTR with CAD from those with stable renal function were 89.3%, 86.4%, 89.3% and 86.4% respectively at a cut-off level of 872 ng/ml (AUC = 0.972, 95% CI 0.937–1.000, $p < 0.001$). The sensitivity, specificity, PPV and NPV of serum hsCRP in discriminating RTR with CAD from those with stable renal function were 71.4%, 68.2%, 74.1% and 65.2% respectively at a cut-off level of 5.27 mg/l (AUC = 0.753, 95% CI 0.609–0.898, $p = 0.002$). Serum sCD163 was superior to serum hsCRP in the detection of CAD after RT (z = 3.192, 95% CI 0.0846–0.354, $p = 0.001$) (Fig. 3).

Stratified analysis for the relationship between serum soluble CD163 levels and renal allograft outcome in renal allograft recipients after adjustment for possible confounding variables

The association between serum sCD163 levels and renal allograft outcome of the whole patient group of RTR showed that the crude (unstratified) OR was 52.778 (95% CI, 9.566–291.185; $p < 0.001$) (Table 5). To assess the confounding effect of other variables, the association between serum sCD163 and renal allograft outcome was adjusted separately for age, sex and anthropometric data. Stratified analysis using the Mantel-Haenszel formula showed the homogeneity of stratum-specific ORs for each variable ($p > 0.05$). When compared with the whole group, confounding was present for sex (adjusted OR = 44.961; 95% CI, 8.136–248.460; $p < 0.001$), BMI (adjusted OR = 30.947; 95% CI, 5.270–181.733; $p < 0.001$) and central obesity (adjusted OR = 42.090; 95% CI, 7.135–248.289; $p < 0.001$) while age (below and equal/above median of 40 years) was not a confounding variable (the difference between one stratum-specific OR from the crude OR was <10%) (Table 6).

Discussion

Macrophage activation plays an important role in acute and chronic kidney diseases [10–14]. The present work showed a

Table 3 Statistical correlations between serum soluble CD163 (sCD163) levels (ng/ml) on one hand and renal function, renal resistive index and degree of renal fibrosis on the other hand in renal transplant recipients (RTR) with stable renal function and chronic allograft dysfunction (CAD).

Variables	Serum sCD163 (ng/ml)			
	RTR with stable renal function (n = 22)		RTR with CAD (n = 28)	
	r	p	r	p
Serum creatinine (mg/dl)	0.643	0.001	0.420	0.026
eGFR (ml/min/1.73m^2)	- 0.375	0.085	−0.419	0.026
Urinary ACR (mg/g)	0.588	0.004	0.452	0.016
Renal resistive index	0.485	0.022	0.623	<0.001
Renal interstitial fibrosis	–	–	0.827	<0.001

Abbreviations: eGFR: Estimated glomerular filtration rate; ACR: Albumin/creatinine ratio.
r: Spearman correlation coefficient.

Table 4 Statistical correlations between serum soluble CD163 (sCD163) levels on one hand and serum high sensitivity C-reactive protein (hsCRP) levels and metabolic parameters on the other hand in renal transplant recipients (RTR) with stable renal function and chronic allograft dysfunction (CAD).

Variables	Serum sCD163 (ng/ml)			
	RTR with stable renal function (n = 22)		RTR with CAD (n = 28)	
	r	p	r	p
Serum hsCRP (mg/l)	0.574	0.005	0.697	<0.001
Body mass index (kg/m^2)	0.500	0.018	0.520	0.005
Waist-to-hip ratio	0.427	0.047	0.504	0.006
Total cholesterol (mg/dl)	0.540	0.009	0.565	0.002
HDL-C (mg/dl)	- 0.617	0.002	−0.579	0.001
LDL-C (mg/dl)	0.549	0.008	0.575	0.001
Triglycerides (mg/dl)	0.619	0.002	0.554	0.002
Fasting plasma glucose (mg/dl)	0.095	0.676	0.111	0.574
Fasting serum insulin (μIU/ml)	0.568	0.006	0.766	<0.001
HOMA-IR	0.587	0.004	0.751	<0.001

Abbreviations: HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance.
r: Spearman correlation coefficient.

significant increase in serum levels of sCD163, a marker of macrophage activation, in RTR, which was more pronounced in patients with CAD and was positively correlated with worsening of renal function, RRI and degree of renal fibrosis. These findings suggest that macrophages are persistently

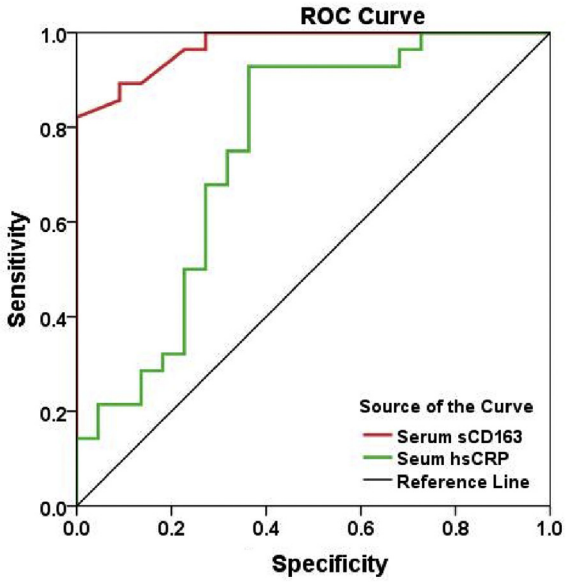

Fig. 3 Receiver operating characteristic curve (ROC) shows that the sensitivity and specificity of serum soluble CD163 (sCD163) and serum high sensitivity C-reactive protein (hsCRP) in discriminating renal transplant recipients (RTR) with stable renal function from RTR with chronic allograft dysfunction were 89.3% and 86.4% respectively at a cut-off level of 872 ng/ml (AUC = 0.972, 95% CI 0.937–1.000, $p < 0.001$) and 71.4% and 68.2% respectively at a cut-off level of 5.27 mg/l (AUC = 0.753, 95% CI 0.609–0.898, $p = 0.002$) respectively.

activated after RT and peaked after the development of allograft dysfunction and IF/TA with increased renal vascular resistance. To our knowledge, this is the first study addressing the association between serum sCD163 levels and renal allograft dysfunction. Previous studies demonstrated increased sCD163 levels in patients with CKD [28], diabetes with worsening renal function [29], immunoglobulin A nephropathy [30] and hemorrhagic fever with renal syndrome [31]. In the renal transplant setting, Guillén-Gómez et al. [32] found that surface CD163 expression on monocytes increased significantly from 1 week after RT and this increase

Table 5 Crude (unstratified) odds ratio (OR) for the association between serum soluble CD163 (sCD163) levels and allograft outcome in renal transplant recipients.

Variable	Renal transplant recipients		
	Stable renal function	CAD	Total
Serum sCD163 (ng/ml)			
<872	19 (86.4)	3 (13.6)	22 (100.0)
≥872	3 (10.7)	25 (89.3)	28 (100.0)
Total	22 (44.0)	28 (56.0)	50 (100.0)
Crude	OR = 52.778 (95% CI, 9.566–291.185; $p < 0.001$)		

Abbreviations: CAD: Chronic allograft dysfunction; CI: Confidence interval.

Table 6 Odds ratio (OR) for the association between serum soluble CD163 (sCD163) levels and allograft outcome in renal transplant recipients after stratification for age, sex and anthropometric measurements.

Variables	Serum sCD163 (ng/ml)	Renal transplant recipients			OR (95% CI)	*p*-value
		Stable renal function	CAD	Total		
Age (years)						
<40	<872	11 (84.6)	2 (15.4)	13 (100.0)	60.500	
	≥872	1 (8.3)	11 (91.7)	12 (100.0)	(4.763–768.485)	
	Total	12 (48.0)	13 (52.0)	25 (100.0)		
≥40	<872	8 (88.9)	1 (11.1)	9 (100.0)	56.000	
	≥872	2 (12.5)	14 (87.5)	16 (100.0)	(4.360–719.205)	
	Total	10 (40.0)	15 (60.0)	25 (100.0)		
Sex						
Male	<872	15 (88.2)	2 (11.8)	17 (100.0)	82.500	
	≥872	2 (8.3)	22 (91.7)	24 (100.0)	(10.442–651.789)	
	Total	17 (41.5)	24 (58.5)	41 (100.0)		
Female	<872	4 (80.0)	1 (20.0)	5 (100.0)	12.000	
	≥872	1 (25.0)	3 (75.0)	4 (100.0)	(0.514–280.089)	
	Total	5 (55.6)	4 (44.4)	9 (100.0)		
Adjusted OR					44.961 (8.136–248.460)	<0.001
Body mass index						
Normal	<872	12 (92.3)	1 (7.7)	13 (100.0)	12.000	
	≥872	1 (50.0)	1 (50.0)	2 (100.0)	(0.384–374.837)	
	Total	13 (86.7)	2 (13.3)	15 (100.0)		
Overweight	<872	7 (77.8)	2 (22.2)	9 (100.0)	42.000	
	≥872	2 (7.7)	24 (92.3)	26 (100.0)	(4.976–354.537)	
	Total	9 (25.7)	26 (74.3)	35 (100.0)		
Adjusted OR					30.947 (5.270–181.733)	<0.001
Central obesity						
No	<872	7 (77.8)	2 (22.2)	9 (100.0)	10.500	
	≥872	1 (25.0)	3 (75.0)	4 (100.0)	(0.668–165.114)	
	Total	8 (61.5)	5 (38.5)	13 (100.0)		
Yes	<872	12 (92.3)	1 (7.7)	13 (100.0)	132.000	
	≥872	2 (8.3)	22 (91.7)	24 (100.0)	(10.820–1610.316)	
	Total	14 (37.8)	23 (62.2)	37 (100.0)		
Adjusted OR					42.090 (7.135–248.289)	<0.001

Abbreviations: CAD: Chronic allograft dysfunction; CI: Confidence interval.

correlated with 4-month creatinine levels. Also, Sekerkova et al. [33] showed that the proportions of CD14+CD163+ monocytes were transiently upregulated early after RT and remained higher during the first month in most patients. Several investigators identified CD163+ M2 phenotype as the major population of macrophages in antibody- or T-cell-mediated rejection [12,34] and chronic kidney allograft injury [13] and found that glomerular CD163+ cells infiltration was correlated with higher serum creatinine levels, lower eGFR and poorer graft function [12,13]. Similarly, macrophage activation has been reported in other solid organ transplantations. Soluble CD163 levels were significantly increased in patients with early liver allograft dysfunction compared with patients with stable allograft function [35]. Moreover, Schreurs et al. [36] found that the expression of CD163 on blood monocytes was significantly increased in chronic lung allograft dysfunction. Also, significantly more CD68+CD163+ macrophages were demonstrated in endomyocardial biopsies during acute heart transplant rejection compared to barely present M1 macrophages [37]. The shedding of CD163 into the circulation where it could be measured, may serve as a clinically relevant biomarker for allograft dysfunction after RT [17].

Macrophages, mainly M2 phenotype, are highly involved in the process of renal injury, repair and fibrosis. During renal inflammation, monocyte-derived macrophages are recruited, activated, and polarized in response to the local microenvironment. Activated M2 macrophages may contribute to kidney repair by exerting anti-inflammation and wound healing functions. However, sustained M2 macrophage activation promotes extracellular matrix deposition leading to renal fibrosis [11], a major driver of graft loss in chronic renal allograft injury [3,4]. Toki et al. [14] found that ninety-two percent of infiltrating macrophages exhibited an M2 phenotype in renal allograft biopsies obtained 1 year after RT, which was associated with subclinical alloimmune inflammation, tubular injury and progression of fibrosis. Also, Shin et al. [34] found that CD163+ infiltrates correlated strongly with interstitial inflammation, tubulitis, and peritubular capillaritis (*ptc*) scores mainly in early biopsies of T-cell-mediated rejection. Similarly, another study demonstrated higher intensity and percentage of CD163-marked cells in the tubulointerstitial compartment was associated with advanced interstitial fibrosis in kidney allograft biopsies during 2 years [38]. Ikezumi et al. [13] showed that CD163+ M2-type macrophages were frequently localized in areas of interstitial fibrosis

exhibiting collagen I deposition and accumulation of myofibroblasts post RT. The depletion of M2 macrophages improved renal fibrosis with the reduction of type IV collagen [11]. M2 macrophages induce renal fibrosis via paracrine effects with the continuous production of wound healing growth factors such as TGF-β [11] or through the direct transition of macrophages to myofibroblast-like cells [39].

Although CD163 is expressed on M2 macrophages, sCD163 may not reflect only M2 macrophage activation, but also the presence of inflammation. The present study showed that the increase of serum sCD163 levels was in parallel with an increase in serum levels of hsCRP, a marker of systemic inflammation. Similarly, previous studies showed a close association between serum levels of sCD163 and hsCRP in obese subjects [22,40] indicating that sCD163 is a biomarker linking macrophage activation with systemic inflammation [18]. Soluble CD163 could reflect inflammation since it is shed from membrane CD163 upon inflammatory activation of macrophages in response to proinflammatory stimuli, such as lipopolysaccharide and IL-6 and ADAM17/TACE, the enzyme cleaving CD163 to become soluble, is expressed by the proinflammatory M1 macrophages. Moreover, the proinflammatory cytokine TNF-α is also shed from the surface of activated macrophages in response to lipopolysaccharide in concomitant with sCD163 by the same enzymatic system. Thus, sCD163 may be regarded as a long-circulating surrogate marker for circulating TNF-α since sCD163 level has a much longer half-life than TNF-α [19]. The present study showed that serum sCD163 was superior to serum hsCRP in the detection of CAD after RT with higher sensitivity and specificity.

Besides its role in graft dysfunction, macrophages play an important role in metabolic derangements [15,16], which are frequent complications after RT often exacerbated by immunosuppressive drugs [5]. The present study showed increased BMI and WHR, dyslipidemia, FSI and IR in patients with RT particularly with the development of CAD and these abnormalities were positively correlated with serum sCD163 levels supporting the link between macrophage activation and metabolic abnormalities. Previous studies showed an association between serum sCD163 concentration and a less favorable metabolic profile as judged by higher BMI, waist circumference, visceral fat [40–42], glucose homeostasis parameters, insulin [43], HOMA-IR [41], TC [43], LDL-C [40] and TG and by a lower HDL-C [42]. Moreover, Hu et al. [42], found that sCD163 levels independently predicted the risk of metabolic syndrome and central obesity and had positive trends with diabetes, dyslipidemia and non-alcoholic fatty liver disease. During lifestyle intervention, the change in sCD163 was positively associated with changes in BMI, FBG [44], HOMA-IR [22,44] and cholesterol, and inversely associated with the change in HDL-C [22] in obese subjects and patients with non-alcoholic fatty liver disease.

The increase in circulating sCD163 in association with various markers of metabolic dysfunction after RT may reflect the presence of an inflammatory state that contributes to the deregulated metabolism of glucose and lipids. Macrophage activation results in enhanced production of inflammatory cytokines in adipose tissue and other metabolic organs, which lead to IR [15]. These cytokines cause a decrease in insulin sensitivity in the insulin target cells (adipocytes, hepatocytes, and myocytes) by inhibiting the insulin receptor tyrosine kinase activity and the insulin receptor substrate 1 phosphorylation and by activating a number of transcription factors such as the suppressors of cytokine signaling, nuclear factor kappa B and serine kinases including JNK and protein kinase C that impair insulin signaling at the insulin receptor and the insulin receptor substrates levels [15]. Moreover, macrophage activation promotes dyslipidemia by stimulating lipolysis in adipocytes leading to increased levels of circulating free fatty acids and their delivery to the liver to increase triglyceride synthesis and reducing lipoprotein lipase activity and the clearance of triglyceride-rich lipoproteins [16]. Also, macrophage activation increases circulating TC and LDL-cholesterol levels by activating cholesterol synthesis and modify the size, composition and function of HDLs, which leads to the impairment of reverse cholesterol transport and parallel changes in apolipoproteins, cholesterol metabolism-related enzymes [16,45]. Obesity is characterized by chronic low-grade inflammation with abundance of infiltrating macrophages in adipose tissue leading to IR and dyslipidemia [15]. Whether immunosuppressive drugs promote metabolic derangements after RT through macrophage activation, is still unclear. In the transplant setting, it is well-established that immunosuppressive agents may exacerbate the risk of post-transplant metabolic complications through direct inhibitory effects on pancreatic β-cell function and insulin sensitivity and by interfering with lipid metabolism [5]. However, an *in vitro* study showed that mycophenolate mofetil shifted macrophage polarization toward an M2-like phenotype and increased the expression of M2 surface markers including CD163 [46].

The association between sCD163 levels and the development of CAD after RT may be influenced by other risk factors. The present study showed that male sex, increased BMI and central obesity, but not age, were potential confounders for this association, although the relative risk of sCD163 for the development of CAD remained significant after further adjustment for these variables. In a long-term retrospective study, male gender was found to be an independent prognostic factor for poor renal transplant survival. Hormonal effects, complex immunological processes and compliance to therapy may explain the better long-term prognosis after RT in women [47]. Also, obesity may represent an independent risk factor for graft loss among RTR. A recent study showed that obese patients displayed an increased incidence of delayed graft function and a higher 1-year serum creatinine after RT compared with nonobese recipients [48]. Potential mechanisms for kidney injury in obesity may include glomerular hyperfiltration, inflammatory processes, hormone activation and the development of other comorbid conditions such as diabetes and hypertension, which may affect kidney function [49].

Conclusions

Based on the results of the present study, it can be concluded that macrophage activation, reflected by increased circulating sCD163, may play a role in the development of CAD and

metabolic derangements after RT. Future studies need to be conducted to assess whether modulation of macrophage activation and polarization could be a therapeutic option for CAD, renal fibrosis and metabolic derangements. The usefulness of serum sCD163 as a potential simple biomarker for detecting allograft dysfunction after RT has to be validated in clinical trials with a large-scale population.

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Conflicts of interest

The authors declare that there is no conflicts of interest regarding the publication of this paper.

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