Macrophage activity assessed by soluble CD163 in early rheumatoid arthritis: association with disease activity but different response patterns to synthetic and biologic DMARDs

S.R. Greisen[1,2], H.J. Møller[3], K. Stengaard-Pedersen[2], M.L. Hetland[4,5], K. Hørslev-Petersen[6], P. Junker[7], M. Østergaard[4,5], M. Hvid[1,8], B. Deleuran[1,2,8]

[1]Department of Biomedicine, Aarhus University, Denmark; [2]Department of Rheumatology, and [3]Department of Clinical Biochemistry, Aarhus University Hospital, Denmark; [4]Copenhagen Centre for Arthritis Research, Centre for Rheumatology and Spine Diseases, Glostrup Hospital, Denmark; [5]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark; [6]King Christian 10th Hospital for the Rheumatic Diseases, and University of Southern Denmark, Denmark; [7]Department of Rheumatology, Odense University Hospital, Denmark; [8]Department of Clinical Medicine, Aarhus University, Denmark.

Abstract

Objective

Rheumatoid arthritis (RA) is a chronic autoimmune disease where TNF-α is a central mediator of inflammation, and is cleaved from the cell surface by TACE/ADAM17. This metalloproteinase is also responsible for the release of soluble (s) CD163. Soluble CD163 reflects macrophage activation. In RA, sCD163 has been suggested as a marker of disease activity and progression. Our aim is to investigate sCD163 levels in early RA patients.

Methods

Soluble CD163 was measured by ELISA from 150 RA plasma samples from the OPERA trial. Averaged disease duration was three months, prior to randomisation with methotrexate (MTX) and adalimumab (DMARD+ADA) or MTX and placebo (DMARD+PLA). Soluble CD163 levels were evaluated in relation to clinical disease parameters.

Results

Plasma sCD163 at baseline was 2.39 mg/l (1.74 mg/l-3.18 mg/l), mean (95% CI), vs healthy controls: 1.63 mg/l (1.54 mg/l – 1.73 mg/l), ($p<0.001$). After three months of treatment sCD163 levels decreased significantly (average 23.5%) in both treatment groups. Significant incremental sCD163 levels followed withdrawal of ADA after 12 months of treatment. Baseline sCD163 correlated with CRP and all investigated disease activity markers ($\varrho=0.16$-0.28, $p<0.05$). In the DMARD+PLA group baseline sCD163 also correlated with CRP during the follow-up period.

Conclusion

Soluble CD163 correlated with disease activity markers in early RA before treatment. Plasma sCD163 may add to currently available disease measures by specifically reflecting changes in macrophage activity as evidenced by increasing levels following anti-TNF withdrawal, despite maintenance of a stable clinical condition achieved by conventional remedies. It remains to be determined whether sCD163 is an early predictor of disease flare

Key words

soluble CD163, early rheumatoid arthritis, biomarker

Stinne Ravn Greisen, MD, PhD
Holger Jon Møller, MD, DMSc
Kristian Stengaard-Pedersen, MD, DMSc
Merete Lund Hetland, MD, DMSc
Kim Hørslev-Petersen, MD, DMSc
Peter Junker, DMSc
Mikkel Østergaard, MD, PhD, DMSc
Malene Hvid, PhD
Bent Deleuran, MD, DMSc

Please address correspondence and reprint requests to:
Stinne Greisen,
Department of Biomedicine,
Aarhus University, Building 1240,
Wilhelm Meyers Allé 4,
8000 Aarhus C, Denmark.
E-mail: srg@biomed.au.dk

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease, mainly affecting the joints, and dominated by T cell and macrophage activity (1). TNF-α is a key player in disease progression, as reflected by the effects of anti-TNF treatment (2). CD163 is a scavenger receptor specifically expressed on macrophages and monocytes, binding haemoglobin-haptoglobin complexes (3). The soluble form of CD163 (sCD163) is present in plasma and other tissue fluids, associated with the inflammatory state, and considered a specific marker of macrophage/monocyte activation (4, 5). Soluble CD163 is shed from the cell surface by TACE/ADAM 17 –the metalloproteinase also responsible for cleaving TNF-α (6), suggesting a close link between sCD163 and TNF-α. The functional role of sCD163 is yet to be determined; an anti-inflammatory role, decreasing lymphocyte proliferation and activity has been suggested, (7) but sCD163 has also been suggested to mediate innate immunity by decreasing iron availability (5). However, sCD163 is especially useful as a marker of immune activation as levels increase in concordance with TNF-α concentrations following macrophage activation. The longer half-life of sCD163 compared with TNF-α enables a more reliable measuring in plasma, favouring sCD163 as an inflammatory biomarker (8, 9). Today many RA patients achieve remission, however, some patients still have progressing disease and others fail to respond to, or tolerate treatment. Hence, in order to identify disease progression early, an on-going search for new biomarkers in RA is of great interest. Previously, we investigated sCD163 in a small cohort of RA patients (10). We observed that sCD163 was correlated with disease activity and could reflect radiographic progression up to five years from treatment initiation. Here, we investigate sCD163 in early treatment naïve RA patients receiving two different treatment regimes.

Materials and methods

Collection of patient samples and clinical data

Serial plasma samples were obtained from a subset of patients (n=154, age=53.5 (51–55), 70% women) randomly selected from the OPERA study (OPtimized treatment algorithm in Early Rheumatoid Arthritis). The trial was conducted in accordance with the Helsinki declaration and approved by the Danish Medical Agency (2612-3393), the Danish Data Protection Agency (2007-41-0072) and the Regional Ethics Committee (VEK-20070008). All patients gave written consent to participation (11). Briefly, upon entry, treatment-naïve early RA (eRA) patients with symptoms for an average of three months were randomised to conventional methotrexate (MTX) treatment plus adalimumab (DMARD+ADA) or MTX plus placebo (DMARD+PLA); both regimes were given in combination with intra-articular glucocorticoid injections. At baseline, IgM-rheumatoid factor (IgM-RF) and anti-CCP (ACPA) were measured. Disease activity was assessed when plasma samples were collected. In this study we used serum C-reactive protein (CRP), number of swollen (SJC 28 and 40) and tender joints (TJC 28 and 40), physicians' global assessment of disease activity on a visual analogue scale (VAS doctor global), simplified disease activity index (SDAI), clinical disease activity index (CDAI), health assessment questionnaire (HAQ), disease activity score in 28 joints (DAS28CRP, four variables, CRP-based) and ACR response 20, 50 and 70. Radiographic progression was assessed by change in Total Sharp Score (TSS) from baseline to one and two years. After one year of treatment, adalimumab was discontinued and patients continued follow up and received treatment aiming at synovitis suppression and DAS28CRP < 3.2 (11). The patients' clinical characteristics are presented in Table I.

Soluble CD163 ELISA

Soluble CD163 was quantified in plasma from the eRA patients (n=150) using a standardised and validated in-house sCD163 ELISA, as previously described (12). The sCD163 levels in 217 healthy controls (HC) (age=56.4 (54-58)) have previously been established using the same assay. As sCD163

Table I. Patients baseline characteristics.

Disease activity marker	DMARD+ ADA	DMARD+PLA
IgM-RF (%positive)	70.0	73.0
ACPA (%positive)	57.9	70.5
CRP (mg/l)	13.5 (7.0-39.3)	15 (7-59)
DAS28CRP	5.7 (4.8-6.5)	5.6 (5.1-6.1)
HAQ	1.1 (0.88-1.9)	1.1 (0.63-1.5)
VAS	58.0 (44.0-76.0)	55.5 (39.8-70.3)
CDAI	34.2 (24.4-43.1)	31.5 (26.4-38.1)
SDAI	35.3 (26.8-46.2)	32.9 (26.8-43.3)
SJC 28	9.0 (6.0-14.0)	8.0 (6.0-13.0)
SJC 40	11.5 (7.3-18.0)	12.0 (8.0-19.3)
TJC 28	11.0 (7.3-17.8)	11.0 (8.0-14.0)
TJC 40	16.5 (10.3-23.8)	16.5 (10.8-22.3)

Patient characteristics at baseline: Data are expressed as median with (IQR). Treatment group methotrexate plus adalimumab (DMARD+ADA), treatment group methotrexate plus placebo (DMARD+PLA), IgM rheumatoid factor (IgM-RF), anti-citrullinated protein antibodies (ACPA), C-reactive protein (CRP), Disease activity score based on CRP (DAS28CRP), Health Assessment Questionnaire (HAQ), physicians' global assessment of disease activity on a visual analogue scale (VAS), Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI), swollen joint count by 28 joints (SJC28) or 40 (SJC40) joints, tender joint count by 28 joints (SJC28) or 40 (SJC40) joints.

is not affected by gender, HC and patients were not gender matched (5).

Statistics

Statistical analyses were performed using Stata13 (TX, USA) and Graphpad Prism (CA, USA). Soluble CD163 data are expressed as mean (95% CI), if not otherwise specified. Correlations were assessed using Spearman rho, as other parameters did not fit the normal distribution. Regression and linear regression were calculated when suitable, where β is the correlation coefficient (95% CI). All $p<0.05$ are considered statistically significant. Soluble CD163 levels were log-transformed in order to fit the normal distribution.

Results

Soluble CD163 was increased in early RA

Plasma levels of sCD163 were increased (2.39 mg/l (1.74 mg/l-3.18 mg/l)) in treatment naïve eRA patients compared with HC ((1.65 mg/l (1.55–1.76 mg/l), $p<0.001$). Soluble CD163 levels decreased significantly after three month of treatment; DMARD+ADA: (1.88 mg/l (1.41–2.35 mg/l), $p<0.001$), and DMARD+PLA: (1.75 mg/l (1.56–1.95 mg/l), $p<0.001$). Simultaneously, DAS28CRP decreased from 5.6 to 2.6 ($p<0.001$). After six months of treatment, sCD163 levels were comparable with HC levels in both treatment groups: DMARD+ADA: (1.68 mg/l (1.52–1.86 mg/l)) DMARD + PLA: (1.75 mg/l (1.58–1.95 mg/l)) (Fig. 1).

Soluble CD163 was affected by the addition of adalimumab

Withdrawal of adalimumab after 12 month was reflected by a marked increase in sCD163 plasma levels from 12 to 24 months; 1.72 mg/l (1.55–1.93 mg/l) to 2.10 mg/l (1.88–2.29 mg/l) ($p=0.0001$), also significantly higher than in HC ($p<0.001$). In the DMARD+PLA group, sCD163 levels did not change from 12 to 24 months; (1.83 mg/l (1.63–2.05 mg/l) and 1.77 mg/l (1.58–1.97 mg/l) respectively, $p=0.4$) (Fig. 1). Adjusting for patients receiving biological treatment in the two groups between 12 and 24 months due to active disease (DMARD+ADA n=14 and DMARD+ PLA n=12) did not influence these findings (data not shown).

Soluble CD163 correlated with disease activity parameters

At baseline, plasma levels of sCD163 correlated with markers of disease activity. Using Spearman's rank correlation test we observed correlations with markers of systemic inflammation, including CRP (ϱ=0.238) and VAS doctor global (ϱ=0.27). We also observed correlations with composite disease activity markers, such as

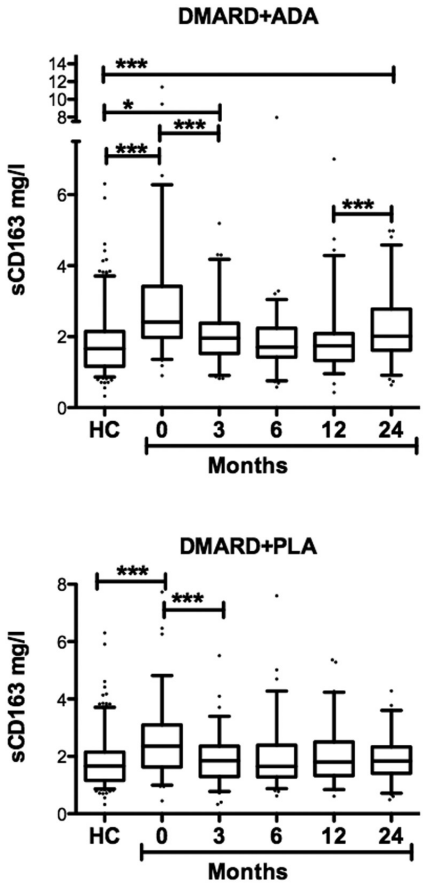

Fig. 1. Plasma levels of sCD163 in the two treatment groups and HC.
Plasma levels of sCD163 in DMARD+ADA (n=73) and DMARD+PLA (n=77) treated patients at baseline and at each follow-up, and plasma levels of sCD163 in age-matched HC (n=240). ***$p<0.0001$; *$p<0.05$.

SDAI (ϱ=0.233), CDAI (ϱ=0.211) and DAS28CRP (ϱ=0.25) and functionality; HAQ (ϱ=0.218) (Table II). We observed no correlations with TSS nor delta-TSS at any time-point, nor with IgM-RF and ACPA.

The association between CRP and sCD163 fitted a linear regression model at baseline (β=8.28, $p<0.001$). In the DMARD+PLA group the association was sustained for one year of treatment (β=9.9, $p<0.001$). In the DMARD+ADA group we only observed this association at baseline and after six months of treatment (Fig. 2).

Soluble CD163 at baseline predicts CRP

We considered if sCD163 could predict future disease activity, and by regression analysis we observed that sCD163 at baseline correlated with CRP in the

Table II. Correlation between disease activity markers and sCD163 at baseline.

Baseline sCD163	CRP	VAS	HAQ	DAS28CRP	SDAI	CDAI	SJC28	SJC40	TJC28	TJC40
ϱ	0.228	0.270	0.218	0.250	0.233	0.211	0.151	0.175	0.135	0.165
(p)	(0.004)	(<0.001)	(0.008)	(0.002)	(0.004)	(0.01)	(0.07)	(0.03)	(0.1)	(0.04)

Correlations between plasma sCD163 and markers of disease activity at baseline: Correlations were assed using Spearman rho. C-reactive protein (CRP), Disease activity score based on CRP (DAS28CRP), Health Assessment Questionnaire (HAQ), physicians' global assessment of disease activity on a visual analogue scale (VAS), Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI), swollen joint count by 28 joints (SJC28) or 40 (SJC40) joints, tender joint count by 28 joints (SJC28) or 40 (SJC40) joints.

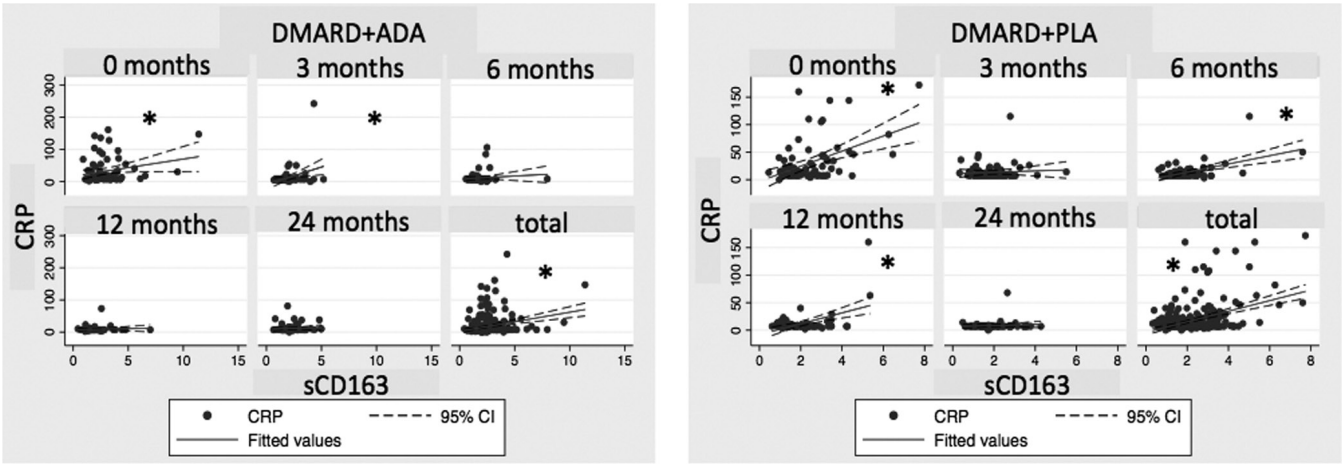

Fig. 2. Linear regression of sCD163 and CRP.
The linear regression model fitted to sCD163 and CRP in the two treatment groups and at each time of follow-up. *regression coefficient with $p<0.05$.

entire follow-up period, but only in the DMARD+ PLA treated group (Table III).

Discussion

Soluble CD163 is an established marker of macrophage activity in inflammatory conditions. Here, we investigated sCD163 in early RA patients with average disease duration of only three months. We observed that sCD163 levels were significantly elevated at baseline, in line with previously published results (10), indicating that elevated sCD163 reflects active inflammatory disease. This is further supported by the positive correlation between baseline sCD163 levels and traditional disease activity markers. Baeten *et al*. (13) also reported elevated sCD163 levels in chronic RA patients. Though they did not observe any correlation with disease activity markers, possibly explained by the chronicity. Thus we only observed baseline sCD163 to correlate with disease activity markers, suggesting sCD163 to be especially valuable as a disease activity marker in early disease. Following three months of treatment sCD163 levels decreased significantly coinciding with decreasing DAS28CRP. At six months sCD163 levels were comparable with those in HC. In line with Baeten *et al*. (10) we did not observe sCD163 levels to be affected by anti-TNF treatment. Though withdrawal of ADA after 12 months was followed by a significant increment of sCD163 by the end of the study period, suggesting increased macrophage activity, and that sCD163 may be a surrogate marker for TNF-α levels. The positive correlation between sCD163 and CRP also supports its association with TNF-α, as TNF-α is one of the main inducers of both inflammation and ADAM17, the enzyme responsi-

Table III. Correlation between sCD163 and CRP in the DMARD + PLA treated group.

	CRP baseline	CRP 3 mo	CRP 6 mo	CRP 1 year	CRP 2 years
sCD163 baseline	β =15.2 SE=6.0	β =7.18 SE=2.25	β =5.31 SE=1.8	β =7.07 SE=2.8	β =3.78 SE=1.09
sCD163 3 mo		NS	NS	NS	NS
sCD163 6 mo			β =7.95 SE=2.8	NS	NS
sCD163 1 year				β =7.8 SE=3.9	β =3.37 SE=1.52
sCD163 2 years					NS

Correlation between plasma sCD163 at baseline and C-reactive protein (CRP) through the follow-up period in treatment group methotrexate plus placebo (DMARD+PLA). Correlations are assessed using the regression model. Where indicated, correlation coefficients are all significant with $p<0.05$.

ble for the cleavage of both TNF-α and CD163 (14, 15). This may also explain the absence of the correlation in the group treated with DMARD+ADA. The association between CRP and sCD163 is not strong, however, supporting the complexity regulating inflammation and macrophage activity in eRA (5) and underscoring a putative independent role for sCD163 as biomarker in RA. Unlike our previous study, we did not find an association between sCD163 and radiographic progression (10). This could be explained by the shorter follow-up period, the very short disease duration at baseline and the aggressive treatment regimes -causing less radiographic progression. To conclude, sCD163 holds promise as a complementary marker of macrophage activation in the evaluation of inflammation in early RA patients. It is weakly associated with traditional markers of disease activity, but predicts future CRP levels, thereby possibly enabling early discovery of on-going inflammation. Considering that withdrawal of ADA was followed by increasing sCD163 levels, it is tempting to speculate that sCD163 may serve as a molecular marker of response or resistance to anti-TNF treatment in RA, which warrants further investigation.

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