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Soluble macrophage-derived CD163 is a marker of disease activity and progression in early rheumatoid arthritis

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ABSTRACT

Objective. *To investigate the expression of the soluble form of the resident macrophage marker CD163 (sCD163) and its association with core parameters for disease activity, including radiographic progression in early rheumatoid arthritis (RA).*

Methods. *In a longitudinal sample set from early RA patients (n=34) we measured plasma levels of sCD163 at initiation of treatment and after 9 months of treatment and correlated levels with disease activity in 28 joints (DAS28), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and total Sharp score (TSS). We also measured plasma levels of sCD163 in 55 healthy volunteers (HV) and in a transverse sample set of chronic (>8 years of disease) RA patients (n=24) and OA patients (n=24) with paired plasma and joint fluid.*

Results. *Early RA patients had significantly higher plasma levels of sCD163 (1.69mg/l (1.42–2.10)) (median (IQR)) at baseline than after 9 months of treatment (1.28mg/l (0.963–1.66), p=0.001), but not significantly changed compared with HV (1.66mg/l (1.22–2.02)). In early RA patients, baseline levels of sCD163, correlated with DAS28, CRP and ESR. Interestingly, sCD163 at 9 months was associated with radiographic progression (TSS) between year 0 and 5 (r=0.468, p=0.02). Levels of sCD163 were higher in RA patients, than in OA patients and higher in SF than in plasma.*

Conclusion. *Plasma levels of macrophage derived sCD163 are associated with disease activity and predict radiographic progression in early RA-patients, supporting that sCD163 may have a role as a biomarker of disease activity and that resident macrophages are important for joint destruction.*

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory joint disease. Progression of the disease is associated with cartilage destruction and synovial inflammation, which subsequently result in malformation of the joint. Macrophages and fibroblasts represent the major producers of pro-inflammatory cytokines in the inflamed joint and are primarily present in the synovial lining, sub-lining layer and cartilage pannus junction (1). The infiltrating macrophages express high levels of HLA-DR, tumour necrosis factor α (TNF-α), interleukin (IL-) 1 and IL-6, all known contributors to inflammation (2). Thus macrophages play an important part in the progression of inflammation and joint destruction, supported by association between synovial macrophage infiltration and radiographic progression.

Macrophages in the joint are described as two subpopulations, M1 and M2 (3). M1 is believed to be the first macrophage at sites of inflammation. M2 macrophages are especially found resident in the joint and functionally attributed to both pro- and anti-inflammatory activity (4-6). M2 is characterised by elevated expression of the scavenger receptor CD163.

CD163 is expressed by macrophages and strongly regulated by external stimuli (7). IL-10, IL-6 and glucocorticoids upregulate CD163 expression, while lipopolysaccharide (LPS), TNF-α and interferon γ (IFN-γ) result in downregulation of its surface expression (8).

CD163 is also found in a soluble form (sCD163) due to ectodomain shedding by TNF-α converting enzyme (TACE), which is also responsible for the release of TNF-α (9). The soluble form is upregulated in plasma during acute inflammation such as sepsis, but also during chronic inflammation as in RA (10). Plasma levels of sCD163 have previously been examined in chronic RA and spondylitis (11, 12).

In this study we investigated the association between sCD163 and core parameters for disease activity as well as joint destruction in early RA patients.

Materials and methods

Collection of samples

A longitudinal set of plasma samples was obtained from the CIMESTRA study, with demographic data listed in Table I (14). The patients were newly diagnosed RA patients, with symptoms for no longer than six months, all corticosteroid naïve at the time of entry (n=34). Plasma samples were

obtained from treatment initiation (day 0) and after 9 months of treatment. The study was a double blinded randomised study, where patients at entry were randomised to conventional methotrexate (MTX) treatment combined with an aggressive regime of intraarticular betamethasone injections, with (n=18) or without (n=16) cyclosporine. The two groups are considered as one since no difference in treatment response after 5 years was observed in the current study, in line with the original study (14). All plasma samples were collected at the outpatient clinic of Aarhus University Hospital. Clinical data were obtained the same day as collection of plasma samples. Radiographic measurements were obtained the day of treatment initiation and again 2, 3, 4 and 5 years after diagnosis. Radiographic scoring was done using total Sharp score (TSS). In this study we made use of disease activity in 28 joints (DAS28), Health Assessment Questionnaire (HAQ), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) all recorded in the CIMESTRA study. At the time of diagnosis, the median DAS28 was 5.2 (4.4–5.9), decreasing to 1.9 (1.4–2.4) at 9 months, indicating a high level of disease activity at the time of diagnosis, and low disease activity after 9 months. A cross-sectional sample set of plasma and synovial fluid (SF) was obtained from chronic RA patients (n=24) at the time of knee arthrocentesis, all with disease duration of 8 years or more (Table I). Patients received classical DMARDs only and prednisolone (n=2). A cross sectional sample set of plasma and SF was also obtained from osteoarthritis (OA) patients (n=24). Plasma samples were obtained from healthy volunteers (HV), age- and gender- matched with the CIMESTRA patients (n=55), (age 56 years (46–64) *vs.* 58 years (52–69) $p>0.20$; gender 62% *vs.* 65% women, respectively). All plasma and SF samples were collected in heparinised tubes and kept at -80˚C until used. All samples were obtained after informed written consent according to the Danish Data Protection Agency, the Local Ethics Committee (project numbers 20050046 and 20060012) and the Declaration of Helsinki.

Table I. Baseline demographic, clinical and serologic data from patients with, early rheumatoid arthritis (RA), chronic RA, osteoarthritis (OA) and healthy volunteers.

Characteristics	Early RA	Chronic RA	OA	Healthy volunteers
Number of participants	34	24	24	55
Age (years)	58 (52–69)	61 (56–71)	68 (62–80)	56 (46–64)
Gender (% females)	65%	75%	70%	62%
MTX (No.)	18	19	0	0
MTX +CyA (No.)	16	0	0	0
Other DMARD (No.)	0	3	0	0
DAS28	5.2 (4.4–5.9)	NA	–	–
RF positive (%)	65%	79%	0	–
Anti-CCP positive (%)	60%	62%	NA	–
CRP (mg/l)	18.9 (7.8–42.1)	48 (16.5-70.0)	All < 8	–
ESR (mm)	19.5 (10.0–44.8)	NA	NA	–
TSS	3.0 (0.0–9.3)	NA	–	–

Values are expressed as medians with interquartile ranges in parentheses. Treatment and clinical data included; MTX: methotrexate, CyA: cyclosporine A, Other DMARD: hydroxychloroquine, sulfasalazine, and prednisolone, DAS28: disease activity score in 28 joints, RF: rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide antibody, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, TSS: total Sharp score, NA: not assessed. Early RA were all corticosteroid naïve and with symptoms less than 6 months. Chronic RA patients all had a minimum of 8 years disease and samples were taken at the time of knee arthrocentesis.

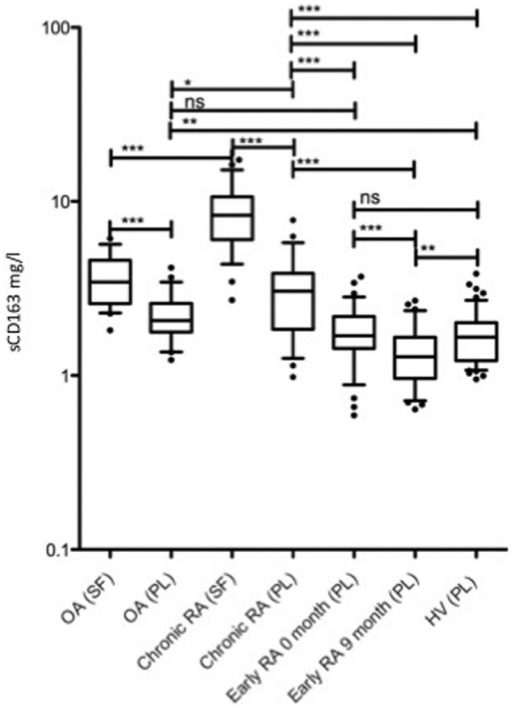

Fig. 1. Plasma (PL) and synovial fluid (SF) levels of sCD163 in healthy volunteers (HV) (n=55), early rheumatoid arthritis (early RA) (n=34), measured at initiation of treatment (0 month) and after 9 months of treatment (9 months), in patients with more than 8 years of disease (chronic RA) (n=24) and in patients with osteoarthritis (OA) (n=24). Bars represent median, IQR and 5-95 percentiles, with the level of significance indicated by $^{*}p<0.01$, $^{**}p<0.001$ and $^{***}p<0.0001$.

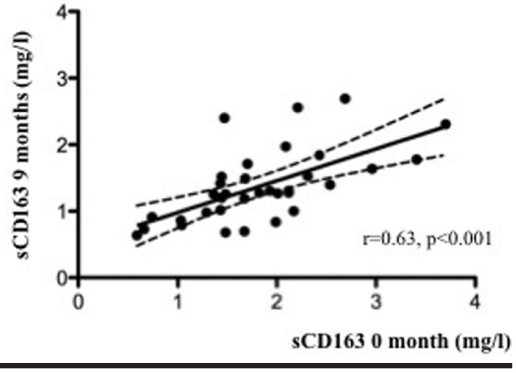

Fig. 2. Plasma levels of sCD163 measured in patients at initiation of treatment (0 month) *versus* after 9 months of treatment, showing a linear association, with dotted lines indicating 5% and 95% level of confidence interval.

Table II. In patients with early rheumatoid arthritis, plasma levels of sCD163 after 9 months of treatment correlated with change in disease progression (TSS) between year 0 and 5, whereas DAS28 did not (*p<0.05).

	sCD163 0 month r (p)	sCD163 9 months r (p)	DAS28 0 month r (p)	DAS28 9 months r (p)
Change in TSS from year 0 – 3	0.177 (*0.36*)	0.258 (*0.18*)	-0.358 (*0.057*)	-0.260 (*0.18*)
Change in TSS from year 0 – 4	-0.07 (*0.75*)	0.260 (*0.23*)	-0.286 (*0.19*)	-0.239 (*0.28*)
Change in TSS from year 0 – 5	0.312 (*0.12*)	0.468 (*0.018*)*	0.005 (*0.98*)	-0.067 (*0.75*)

ELISA

sCD163 was measured in sandwich ELISA essentially described in detail (15).

Statistics

Statistical analyses were performed using GraphPad Prism 5.0 for Mac (GraphPad Software, La Jolla, CA). All graphic data are expressed as medians with interquartile ranges (IQR) and 5 to 95 percentiles. All data in text are expressed as medians with IQR in parentheses. Grouped analyses were done by the Kruskal-Wallis test with Dunn's multiple comparison test as a post-hoc analysis. Evaluation of paired samples was done by Wilcoxon's matched paired test, whereas non-paired data were examined by Mann-Whitney U-test. Correlation was tested using Spearman's Rho. In all tests the level of significance was a two-sided p-value of less than 0.05.

Results

Plasma levels of sCD163 are elevated in chronic RA, but not in early RA

In the longitudinal sample set of newly diagnosed RA patients we measured plasma levels of sCD163 at baseline (1.69 mg/l (1.42–2.10)) and after 9 months of treatment (1.28 mg/l (0.963–1.66)) (Fig. 1). Plasma levels of sCD163 were significantly higher at baseline than after 9 months of treatment, and a strong linear association between plasma levels at baseline and after 9 months was observed (Fig. 2). The sCD163 plasma levels were not increased at the time of diagnosis compared with HV (1.66 mg/l (1.22–2.02)) whereas treatment resulted in significantly lower sCD163 levels compared to HV (Fig. 1).

Plasma levels of sCD163 in chronic RA patients were also measured and found significantly increased (3.05mg/l (1.84–6.04) p<0.001) (Fig. 1), with sCD163 levels in SF elevated to 8.32mg/l (6.04–10.65). Levels of sCD163 in SF and plasma were associated (r=0.4, p=0.05). Plasma and SF levels of sCD163 in chronic RA patients were also found significantly higher than in OA (plasma (2.07mg/l (1.78–2.59)) and SF (3.44mg/l (2.59–4.60)), which again were found increased compared with HV (Fig. 1).

Plasma levels of sCD163 are associated with parameters for disease activity and disease progression in early RA

In early RA patients, plasma levels of sCD163 correlated to CRP (r=0.357, p=0.038), ESR (r=0.536, p=0.0018) and DAS28 (r=0.464, p=0.0057) at baseline. After 9 months of treatment correlation was still observed to CRP (r=0.375, p=0.034) and ESR (r=0.627, p<0.001). We also examined for association between plasma level of sCD163 and change in radiographic score (Delta TSS) revealing a strong association between radiographic progression from year 0 to 5 and sCD163 at 9 months (r=0.468, p=0.018) whereas no association to DAS28 was observed (Table II).

Discussion

In this study we demonstrate that in patients newly diagnosed with RA, plasma levels of sCD163 reflects both disease activity and radiographic progression. Our results suggest that if the immunosuppression is not adequate within 9 months in early RA, this is associated with disease progression, as measured by delta TSS at 5 years.

CD163 is solemnly expressed by macrophages and is cleaved by TACE, which also cleaves membrane bound TNF-α, IL-6R and several other bioactive peptides. However, clearance of these substances often occurs rapidly and it is therefore not adequately reflected in plasma samples. Soluble CD163 has a plasma half-life of up to 24 hours (9), making it a more suitable tool for the study of both macrophage activity and inflammation involving the promiscuous enzyme TACE, especially in TNF-dependent diseases.

Failure to prevent destruction of the inflamed joint is still the major problem in RA, with erosion of the subchondral bone as the main manifestation. Local osteoclastogenesis is the key player, taking place very early in the disease, and is strongly dependent on pro-inflammatory peptides, such as TNF and RANKL, and thereby linked to macrophage activity. We are of the opinion that our results support the importance of controlling macrophage activity in early phases of RA, if future joint destruction should be avoided.

Plasma level of sCD163 in early RA is associated with radiographic progression over years, which supports a possible relation between sCD163 plasma levels and osteoclast activity. This indicates that plasma level of sCD163 is a predictor of disease progression, and when examining the association between DAS28 and radiographic progression, our study suggests, that sCD163 could be a potential biomarker in predicting progression of joint inflammation and disease (16).

Macrophages expressing CD163 are often referred to as M2 and are believed to have both pro- and anti-inflammatory activity (4-6). Even though early RA does not reveal elevated levels of sCD163 compared to what is observed in HV, the close association between plasma sCD163 and CRP, and the other markers of disease activity, supports that these M2 macrophages are indeed pro-inflammatory in RA. The high levels of sCD163 in chronic RA, but not in OA, support the close association of sCD163 to the degree of inflammation.

In conclusion, we have shown that patients with early RA have normal levels of sCD163 that are associated with core parameters of disease activity, and plasma levels of sCD163 examined 9 months after treatment initiation seems to reflect progression of joint destruction years in advance.

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