Plasma sLOX-1 is a potent biomarker of clinical remission and disease activity in patients with seropositive RA

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Abstract

Objectives: Soluble lectin-like oxidized low-density lipoprotein receptor 1 (sLOX-1) is present in the circulation and synovial fluid in patients with rheumatoid arthritis (RA). The aim of this study was to assess whether sLOX-1 level is associated with clinical remission and disease activity in patients with RA.

Methods: Clinical and laboratory data were analyzed for 282 patients with RA. Plasma sLOX-1 level was measured by enzyme-linked immunosorbent assay (ELISA). The remission status and sLOX-1 levels were compared between four groups of patients based on the positivity of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs). Relationships between sLOX-1 level and the 28-joint Disease Activity Score with erythrocyte sedimentation rate (DAS28-ESR) were analyzed by multivariate logistic regression.

Results: The patients in the RF + ACPA + group tended to exhibit higher sLOX-1 levels when compared to the other three groups. In the RF + ACPA + group, the sLOX-1 level was significantly higher in the non-remission group than in the remission group, irrespective of treatment. Multivariate logistic regression showed significant correlations between sLOX-1 level and DAS28-ESR.

Conclusions: sLOX-1 level might be a useful biomarker for assessing clinical remission and disease activity in double-positive RA patients.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that is characterized by synovial inflammation and articular erosion that can progress to joint destruction and physical disability. Biological agents that target inflammatory cytokines can improve the clinical outcomes and physical function of patients with RA. Such anti-cytokine therapies have revolutionized the treatment of RA patients [1]. Treat-to-target treatment, a recent strategy aimed at inducing clinical remission, generally leads to significant remission of early RA. Nevertheless, some patients treated with these therapies remain refractory, and the remission rates achieved have not been as high as expected [2]. Numerous attempts have been made to improve remission rates using various anti-cytokine treatments, but success has remained elusive. This suggests that, although various cytokines undoubtedly play important roles in the inflammation associated with RA, another inflammatory cascade besides the cytokine pathway is involved in the pathogenesis of RA.

Oxidized low-density lipoprotein (LDL) may be involved in another type of inflammatory cascade that occurs during RA. Oxidized LDL is produced by the oxidation of LDL at sites of inflammation [3,4]. Lectin-like oxidized LDL receptor-1 (LOX-1) is an oxidized LDL receptor that is expressed on various cells, including endothelial cells, monocytes, chondrocytes, and fibroblast-like synoviocytes shown by us and others [5–8]. Although basal LOX-1 expression is very low, its expression can be dynamically upregulated by various pathophysiological stimuli such as inflammatory cytokines and oxidized LDL itself [5,9,10]. Activated LOX-1 increases the release of matrix metalloproteinase (MMP) and the expression of adhesion molecules and chemokines [11,12]. Previously, we have shown that the expression of oxidized LDL and LOX-1 protein is increased markedly in the RA synovium and that anti-LOX-1 treatment can prevent synovial inflammation and cartilage destruction in vivo [7,8,13]. These results suggest that the oxidized LDL–LOX-1 axis plays a pivotal role in RA pathogenesis. LOX-1 is cleaved on the cell surface and is shed into the circulation as soluble LOX-1 (sLOX-1) [14,15]. The increased plasma sLOX-1 level reflects increased LOX-1 expression and the extent of disease activity in vascular diseases, such as atherosclerosis and diabetes [16,17].

We have recently found that plasma sLOX-1 level is significantly higher in RA patients than in healthy controls and correlates positively with other inflammatory markers and the extent of RA disease activity [13], suggesting that a high titer of sLOX-1 may be attributed to persistent inflammation. We showed previously that the sLOX-1 level correlated with disease activity in a small cohort of RA patients. The primary aim of this study was to determine whether sLOX-1 level correlates with clinical remission and disease activity in patients with RA in a large cohort.
Materials and methods

Patients

The analyses in the current study were performed using the Kyoto University Rheumatoid Arthritis Management Alliance (KURAMA) cohort database and were approved by the Kyoto University Hospital Ethical Committee. The KURAMA cohort was established in May 2011 at the Center for Rheumatic Disease at Kyoto University Hospital, which aimed at providing strict control of RA and to use patient clinical and laboratory data for clinical investigations [18,19]. Informed consent for this study was obtained from all participating patients. In 2012, a total of 384 patients with RA were enrolled in an annual RA survey. Blood samples were also collected, and the samples for this study were available for 282 of the 384 patients enrolled in the survey. All patients fulfilled the criteria for RA classification according to the American College of Rheumatology revised criteria in 1987 or the American College of Rheumatology/European League Against Rheumatism criteria in 2010.

We extracted the laboratory and clinical variables from the KURAMA cohort database at the time of examination. These data included age, sex, disease duration, swollen, and tender joint counts (SJC and TJC, respectively), C-reactive protein (CRP) concentration, erythrocyte sedimentation rate (ESR), Health Assessment Questionnaire disability (HAQ) index, the titers of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs), 28-joint Disease Activity Score with ESR (DAS28-ESR), and a simplified disease activity index (SDAI). Autoantibody titer levels were considered positive at >12 U/mL for RF and at >4.5 U/mL for ACPAs. Standardized radiographs of the hands/wrists and the feet (posteroanterior view) were obtained at the time of examination and analyzed by two experienced rheumatologists using the van der Heijde-modified Total Sharp Score method (mTSS) [20].

Analysis of patients grouped by disease activity

Clinical remission was defined using the disease activity score on the DAS28-ESR. The patients were separated into two groups based on remission status: a remission group of those with a DAS28-ESR <2.6 and a non-remission group of those with low, moderate, or high disease activity and a DAS28 ≥ 2.6.

Enzyme-linked immunosorbent assay (ELISA) for sLOX-1

Plasma was isolated from blood samples and treated as previously described [13]. The circulating sLOX-1 level in the plasma was measured in duplicate using a CircuLex Human soluble LOX-1 ELISA kit (MBL International, Nagoya, Japan) according to the manufacturer’s instructions. As controls, we examined sLOX-1 level in patients with osteoarthritis (OA) using this kit and found the average was 5.98 ± 10.0 pg/ml (mean ± SD, 0–20.5).

Statistical analyses

Data are presented as the mean ± standard deviation. The values for two groups were compared using the Mann–Whitney U test. Two-way analysis of variance was used to identify differences in the expression of sLOX-1 between groups. The Spearman rank correlation coefficient was used to assess the relationship between the sLOX-1 level and other clinical variables. The clinical variables (independent variables) that were significantly associated with remission according to the DAS28-ESR or sLOX-1 level in the univariate analysis were used in the multivariate logistic regression models. These included age, disease duration, CRP concentration, MMP3 concentration, RF titer, ACPA titer, HAQ index, and mTSS. Significant variables entered in the multivariate logistic regression model were selected using a backward stepwise selection.

Results

Patient characteristics

The baseline characteristics of the patients with RA in this study are summarized in Table 1. This cohort was predominantly female (84.8%) with a mean age of 63.5 years. Of the 282 RA patients, 231 (81.9%) were ACPA positive and 228 (80.9%) were RF positive. Most patients (206, 72.7%) had been treated with methotrexate, and 89 (31.6%) had been treated with biological disease-modifying anti-rheumatic drugs (bDMARDs) including etanercept, infliximab, adalimumab, golimumab, tocilizumab, certolizumab, and abatacept.

Effect of drug treatment on plasma sLOX-1 level

We first examined whether any treatment affected the plasma sLOX-1 level. No correlation was found between plasma sLOX-1 level and the use of prednisolone, methotrexate, or biological DMARDs at the time of examination (Supplementary Figure 1).

Predictive factors for clinical remission in the cohort

We examined whether the sLOX-1 level correlated with other clinical parameters (Table 2). Plasma sLOX-1 level correlated significantly with age, disease duration, stage, class, CRP, ESR, SJC, DAS28-ESR, SDAI, and mTSS, but not with MMP3 or TJC. The sLOX-1 level correlated strongly with the autoantibody titer (RF, ρ = 0.74; ACPAs, ρ = 0.29).

Table 1. Demographics and baseline characteristics.

| Parameter | Value |
|-----------|-------|
| Age (SD) (years) (min–max) | 63.5 ± 13.0 (25–92) |
| Females (n [%]) | 239, 84.8 |
| RA duration ± SD (year) (min–max) | 14.0 ± 11.5 (0–64) |
| Corticosteroid use at baseline (% dose [mg]) (min–max) | 40.8 ± 5.1 ± 3 (0–18) |
| MTX use at baseline (% dose [mg]) | 73.4 (7.2 ± 3.2) |
| Biologics use at baseline (n [%]) | 89 (31.6) |
| RF positive (n [%]) | 228, 80.9 |
| Mean titer ± SD (IU/ml) (min–max) | 89.4 ± 152.2 (0–1020.8) |
| ACPA positive (n [%]) | 231, 81.9 |
| Mean titer ± SD (U/ml) (min–max) | 1208 ± 112.3 (0–300) |
| ESR (mm/h) (min–max) | 26.8 ± 22.4 (1–115) |
| CRP (mg/dl) (min–max) | 0.67 ± 1.3 (0–6.5) |
| TSS (min–max) | 110.6 ± 105.7 (0–443) |
| Full HAQ (min–max) | 0.87 ± 0.8 (0–3) |
| DAS28-ESR (min–max) | 3.19 ± 1.2 (0–6.6) |
| SDAI (min–max) | 8.47 ± 6.6 (0.1–38.6) |

Data are mean ± SD. MTX, methotrexate; RF, rheumatoid factor; ACPA, anti-cyclic citrullinated peptide; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; TSS, total sharp score; HAQ, Health Assessment Questionnaire disability index; DAS-ESR, disease activity score using a 28-joint count and ESR; SDAI, simplified disease activity index (SDAI).
ACPA–, RF + ACPA–, RF–ACPA+, and RF + ACPA+. Of the 282 patients, 28 were negative for both autoantibodies (RF–ACPA–), 23 were positive for only RF (RF + ACPA–), 26 were positive for only ACPAs (RF–ACPA+), and 205 were positive for both autoantibodies (RF–ACPA– or RF–ACPA+ patients) (Figure 1(B)). Plasma sLOX-1 level was significantly higher in the RF + ACPA + patients than in the RF–ACPA– or RF–ACPA + patients ($p < 0.001$ for both comparisons) (Figure 1(B)).

Finally, we examined the contribution of sLOX-1 level and the presence of autoantibodies to clinical remission in the RF + ACPA + patients. Plasma sLOX-1 level was significantly higher in the non-remission group than in the remission group ($p < 0.001$). By contrast, sLOX-1 levels and clinical remission did not differ between the other three subgroups. Plasma sLOX-1 level was significantly associated with SJC, DAS28-ESR, SDAI, and RF titer (Supplementary Table 1). Similar to the observation in the total cohort, the treatment received in the double-positive patients at the time of examination was not related to the plasma sLOX-1 level (Supplementary Figure 2).

**Correlations between disease activity and sLOX-1**

To examine further the relationship between sLOX-1 and disease activity, we performed a stepwise logistic regression analysis. To identify variables that predicted the plasma sLOX-1 level, we selected laboratory and clinical variables that were significant in the univariate analysis (Table 2). In the multivariate analysis, CRP concentration, RF titer, and DAS28-ESR remained significant independent variables (Table 3). We also examined the independent predictors of a high DAS28-ESR score using nine variables, including sLOX-1 level. CRP concentration, HAQ index, and sLOX-1 level remained significant independent variables (Table 4). Taken together, these data suggest that sLOX-1 level is closely related to RA disease activity and seropositivity.

**Discussion**

Because of its pivotal role in amplifying local and systemic inflammation [5,12], the LOX-1–sLOX-1 pathway has generated interest as a potential diagnostic and treatment target for various

![Figure 1](image-url)

**Figure 1.** sLOX-1 levels in the non-remission group were significantly higher than those in the remission group (A). sLOX-1 levels in the autoantibody-double positive patients (RF and ACPAs) were remarkably higher than those in the RF–ACPA- or RF–ACPA+ patients (B). In the double positive (RF + ACPAs+) patients, the sLOX-1 levels in the remission group were significantly higher than those in the non-remission group (C). sLOX-1; Soluble lectin-like oxidized low-density lipoprotein receptor 1, RF; rheumatoid factor, ACPAs; anti-citrullinated protein antibodies, PSL; prednisolone, MTX; methotrexate, BIO; bDMARD.
inflammatory diseases, including cardiovascular diseases and diabetes mellitus [17,21]. The current understanding of the molecular mechanisms underlying RA pathology is that autoantibodies are strongly involved in RA pathogenesis. Thus, the presence of autoantibodies (RF and ACPAs) was included in the new criteria for RA classification in 2010. A high antibody titer indicates a more severe disease course involving joint destruction and reduced likelihood of remission [22,23]. In the present study, we found that circulating sLOX-1 level was significantly higher in the autoantibody-positive patients compared with the negative patients (Figure 1B).

Mitsuoka et al. [24] reported that interleukin 18 (IL-18) increases the sLOX-1 level by increasing LOX-1 cleavage and that a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) is involved in this process. The IL-18 level is increased in RA serum and synovial fluids, and ADAM10 is also overexpressed in RA synovial tissues [24]. Other studies have also reported that IL-18 levels are increased in the serum and synovial fluid of RA patients [25] and that ADAM10 expression is significantly elevated in RA synovial fibroblasts [26]. Thus, the abundance of enzymes that cleave LOX-1 and increase its shedding in patients with RA may be one reason why sLOX-1 level is increased markedly in patients. We also found that the sLOX-1 level correlated with the titer of RF or ACPAs; the RF titer in particular correlated strongly with the sLOX-1 level. Because B cells reportedly express LOX-1 and may be programmed to promote class-switched antibody responses [27], LOX-1 expression might be involved in the production of autoantibodies in patients with RA or vice versa.

It has long been established that the presence of ACPAs is an independent risk factor for joint erosion [28,29]. Interestingly, patients positive for both RF and ACPAs seem to exhibit more severe erosive bone damage compared with those who are positive for only one of the autoantibodies [30], suggesting that the combination of these autoantibodies is strongly linked to a worse clinical disease course of RA [31]. Moreover, we recently reported that RF and ACPAs positivity contribute to joint destruction of particular joints in patients with RA [32,33]. In this study, we found that the sLOX-1 level was markedly higher in patients with the combined presence of RF and ACPAs in the non-remission group compared with those in the remission group (Figure 1C). These results suggest that it may be more difficult for double-positive (RF + ACPA+) patients with a higher sLOX-1 level to achieve remission. A possible mechanism to explain this is that LOX-1 pathway-derived inflammation persists [5,10,34] in patients with higher disease activity and that LOX-1 expression is easily upregulated by cytokines and various other stimuli such as oxidized-LDL, which is a major ligand for LOX-1. Thus, measurement of the sLOX-1 level may have a potent value in assessing double-positive RF + ACPA+ patients.

Although several laboratory markers have shown promise for detecting RA disease activity when used in combination [35,36], a single reliable biomarker for assessing clinical remission has not been established. In this study, we found that plasma sLOX-1 level was significantly higher in the non-remission group than in the remission group but that the sLOX-1 level was not associated with the treatment at the time of examination. Moreover, multivariate logistic regression showed that the sLOX-1 level correlated positively with the DAS28-ESR score. Because sLOX-1 production is easily upregulated by various stimuli [11,12], measuring plasma sLOX-1 level may help to detect even a small amount of inflammation. One concern of the measurement of sLOX-1 would be that, because most of RF– group showed low sLOX-1 value, the presence of RF may interfere with the ELISA system of sLOX-1.

### Table 3. The multivariate analysis in the cohort; stepwise covariate elimination of the influence of disease duration, CRP, ESR, MMP3, RF, ACPAs, Full HAQ, TSS, sLOX-1 to DAS-ESR score.

| Dependent variable | Full model (p value) | First reduced model (p value) | Second reduced model (p value) | Third reduced model (p value) | Fourth reduced model (p value) |
|--------------------|----------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|
| Disease duration   | (p = 0.99)           | CRP (p = 0.032*)              | CRP (p = 0.031*)               | CRP (p = 0.026*)              | CRP (p = 0.005*)              |
| CRP (p < 0.001*)   | ESR (p = 0.28)       | ESR (p = 0.28)               | ESR (p = 0.26)                | ESR (p = 0.26)               | ESR (p = 0.005*)              |
| RF (p < 0.001*)    | ACPA (p = 0.6)       | ACPA (p = 0.6)               | RF (p < 0.001*)               | RF (p < 0.001*)              | RF (p < 0.001*)               |
| RF (p = 0.008*)    | DAS28-ESR (p = 0.6)  | DAS28-ESR (p = 0.6)          | DAS28-ESR (p = 0.007*)         | DAS28-ESR (p = 0.014*)        | DAS28-ESR (p < 0.001*)         |
| RF (p = 0.008*)    | TSS (p = 0.019)      | TSS (p = 0.086)              | TSS (p = 0.095)               | TSS (p = 0.098)              | TSS (p = 0.005*)              |

### Table 4. The multivariate analysis in the cohort; stepwise covariate elimination of the influence of age, disease duration, CRP, MMP3, RF, ACPAs, Full HAQ, TSS, sLOX-1 to DAS-ESR score.

| Dependent variable | Full model (p value) | First reduced model (p value) | Second reduced model (p value) | Third reduced model (p value) | Fourth reduced model (p value) |
|--------------------|----------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|
| Age (p = 0.82)     | Disease duration     | CRP (p < 0.001*)              | CRP (p < 0.001*)               | CRP (p < 0.001*)              | CRP (p < 0.001*)              |
| CRP (p < 0.001*)   | MMP3 (p = 0.388)     | MMP3 (p = 0.368)              | MMP3 (p = 0.368)               | MMP3 (p = 0.346)              | MMP3 (p = 0.346)              |
| RF (p = 0.416)     | ACPA (p = 0.748)     | RF (p = 0.446)                | Full HAQ (p < 0.001*)          | Full HAQ (p < 0.001*)         | Full HAQ (p < 0.001*)         |
| Full HAQ (p < 0.001*) | TSS (p = 0.725)   | sLOX-1 (p = 0.023*)           | sLOX-1 (p = 0.023*)            | sLOX-1 (p = 0.023*)           | sLOX-1 (p = 0.023*)           |
Our preliminary results show that samples of patients with similar levels of sLOX-1 and various levels of RF did not show any trend by the different volume of plasma in this ELISA when added a known level of sLOX-1 recombinant (data not shown). Therefore, concentrations of RF would not directly interfere with sLOX-1 levels in this ELISA system, but this notion requires further analyses. Taken together, sLOX-1 level may be a biomarker for assessing clinical remission of RA, although this idea should be confirmed in a longitudinal study in a larger cohort.

In the previous study, we reported that the sLOX-1 level is correlated with serum level of MMP-3. However we could not find any correlation with them in this study. One possible reason is the difference of biologics usage between the previous and the present cohort. The 31.6% patients in the KURAMA cohort used biologics at baseline, while none of the patients in the previous study used biologics. Biologics therapy can suppress production of MMPs, and, therefore, this difference might have influenced the correlation between production of the MMP-3 and sLOX-1 levels.

This study has several limitations. First, the mechanisms of the close relationship between seropositivity and sLOX-1 level were not pursued in this study. Further immunological in vitro studies would be needed. Second, the average sLOX-1 level of the RA patients in this study (581.3 ± 54 pg/mL) was higher than that reported in our previous study (189.3 ± 36 pg/mL) [13]. The reason may relate to the use of different ELISA kits and the populations examined. Third, changes in remission and disease activity were not investigated because the data were collected in a cross-sectional study. We could not conclude whether sLOX-1 level can predict RA disease activity over time. Further work in a longitudinal cohort study is needed to confirm that sLOX-1 level changes with disease activity. Fourth, we examined remission and disease activity as the primary outcomes, but joint destruction or remission according to the HAQ index may be more important as primary outcomes; these also require a longitudinal study. Finally, the effects of various treatments on plasma sLOX-1 level should be investigated in a larger number of patients over a longer time span.

In conclusion, in the RF + ACPA + patients, sLOX-1 level was significantly higher in the non-remission group than in the remission group, irrespective of treatment. Disease activity was closely related to plasma sLOX-1 level in the multivariate analyses. These results suggest that clinical remission may be difficult to achieve in double-positive (RF + ACPA+) patients with a high plasma sLOX-1 level. sLOX-1 may be a biomarker for assessing the likelihood of clinical remission in these double-positive patients.

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Ethical approval for this study was granted by the committee of Kyoto University Graduate School and Faculty of Medicine (E1308).

Conflict of interest
M.I., M.F., M.H. and T.F. are affiliated with a department that is supported financially by four pharmaceutical companies (Mitsubishi-Tanabe, Bristol-Myers, Chugai, Eisai). H.I. has received grant and research support from Mitsubishi-Tanabe, Chugai, Pfizer, Astellas, and Daiichi Sankyo. A.O., T.M., and S.M. have declared that no conflict of interest exists. The sponsors were not involved in the study design; in the collection, analysis, interpretation of data; in the writing of this manuscript; or in the decision to submit the article for publication. The authors, their immediate families, and any research foundations with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

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Supplementary material available online
Supplementary Table 1
Supplementary Figures 1 and 2