Association between increased serum IL-23 levels and ACPA positivity in patients with rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by the positivity of various antibodies (1), the most specific being autoantibodies to citrullinated antigen (ACPA; anti-citrullinated protein antibody). ACPA-positive individuals are at increased risk of developing RA with a more severe erosive phenotype compared with those with seronegative RA (2, 3).

Interleukin (IL)-23, secreted by activated dendritic cells and macrophages, is an inflammatory cytokine belonging to the IL-12 cytokine family (4). It is essential for the differentiation of naive $CD4^+$ T cells to T helper 17 lymphocytes associated with the inflammation induction in autoimmune tissues and the production of IL-17 and several other inflammatory cytokines (5).

In this study, we measured the serum levels of 12 macrophage-derived cytokines in untreated early RA patients to identify cytokines associated with ACPA positivity in RA. The study was conducted from July 2018 to January 2021 at Hiroshima University Hospital and collaborating institutions. The participants were newly diagnosed RA patients who met the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria (6) and had not previously received disease-modifying anti-rheumatic drugs (DMARDs) or biologics. The patients' serum samples were collected after obtaining written informed consent. The serum concentrations of the following macrophage-derived proinflammatory cytokines were measured at baseline: Th2-type CC chemokine thymus and activation-regulated chemokine (CCL17), CXC motif chemokine 10 (CXCL10), tumour necrosis factor-α, IL-1β, IL-4, IL-6, IL-10, IL-12p70, IL-12p40, IL-23, interferon-γ, and arginase. The Institutional Ethics Committee of Hiroshima University approved the study protocol (permission number E-1087). Sera from 10 healthy donors, with the median age (range) of 43 (31–65) years old, were also collected as controls. Of the 75 RA patients (72 (64–77) years old) analysed, 31 were ACPA-negative and 44 were ACPA-positive (Supplementary Table S1). Serum IL-6 levels were significantly higher in the RA patients than in healthy participants ($p<0.0001$), but not different between the ACPA-positive and ACPA-negative groups (Fig. 1A). Among the 12 cytokines, only the IL-23 levels were significantly higher in the ACPA-positive group than in the ACPA-negative group (median (IQR) 0.99 (0.69–1.77) *vs.* 0.54 (0.50-1.09) pg/mL; $p=0.0013$). At the optimal cutoff (Youden index) of 0.73 pg/mL, the AUC (area under the curve) of the ROC (receiver operating characteristic) curve for predicting ACPA positivity by IL-23 was 0.74 (95% CI 0.62–0.86; $p=0.0004$) with 67.7% (95% CI 48.6–83.3) sensitivity, 75% (95% CI 59.7–86.8) specificity, 2.71 positive likelihood ratio, and 0.43 negative likelihood ratio to identify ACPA positivity (Fig. 1A-B). Serum IL-23 levels did not correlate with disease activity measured by DAS28 and ESR (Fig. 1C).

This is the first report of a significant association between IL-23 and ACPA positivity in patients with untreated early RA. Our results were consistent with those of previous reports showing higher serum IL-23 levels in RA patients compared with a healthy population (4). Moreover, support-

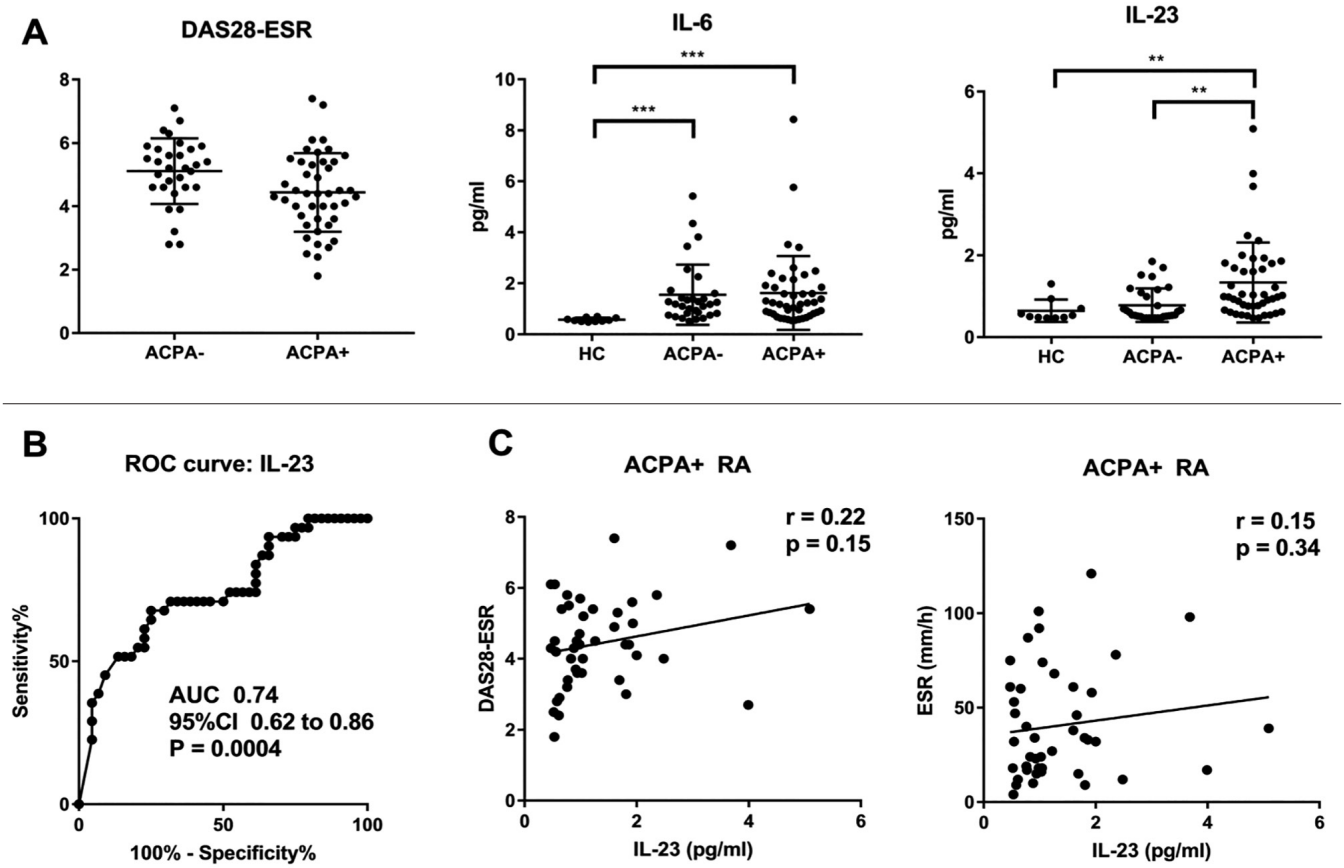

Fig. 1. A: DAS28-ESR at diagnosis in ACPA-negative/positive rheumatoid arthritis (RA) groups. Comparison of IL-6/IL-23 concentrations at diagnosis between healthy controls (HC) and ACPA-negative/positive groups. Differences between the groups were calculated using Kruskal-Wallis test, Dunn test. (*$p<0.05$, **$p<0.001$, ***$p<0.0001$)
B: ROC curve for baseline serum IL-23 in ACPA-negative RA and ACPA-positive RA.
C: Correlation of serum IL-23 and DAS28-ESR/ESR at diagnosis in all RA patients. Spearman's rank correlation coefficient. ACPA: anti-citrullinated protein antibody; AUC: area under the curve; DAS28: Disease Activity Score-28; ESR: erythrocyte sedimentation rate; IL: interleukin; ROC; receiver operating characteristic.

ing the controversial association between IL-23 and disease activity (7, 8), our results showed no association between the disease activity of RA and IL-23. Of note, there was no difference in serum IL-23 levels of the ACPA-negative RA patients even with high disease activity compared with the healthy participants. Recently, the pathogenic role of IL-23 on autoantibody in collagen-induced arthritis models was reported (9). IL-23-activated Th17 cells act on newly differentiated plasmablasts to downregulate the expression of β-galactoside α 2,6-sialyltransferase, thereby diminishing glycosylation of newly produced autoantibodies, unlocking their inflammatory potentials and triggering the onset of arthritis. Considering the action of IL-23, our results suggest that IL-23 may be a useful biomarker for preclinical RA. Due to the small sample size of this study, further studies are needed to draw definitive conclusions on the role of IL-23 in RA pathogenesis.

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