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IL-33 and soluble ST2 levels as novel predictors for remission and progression of carotid plaque in early rheumatoid arthritis: A prospective study

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ABSTRACT

Objectives: To study the association between the baseline IL-33 and soluble ST2 (sST2) levels with disease remission and progression of carotid atherosclerosis in early rheumatoid arthritis (ERA) patients.
Methods: A total of 98 ERA patients were enrolled. Disease activity and the presence of carotid plaque were evaluated at baseline and 12 months later. Plasma IL-33 and sST2 levels were determined using enzyme-linked immunosorbent assay kits.
Results: Baseline IL-33 and sST2 levels were associated with inflammatory markers and cardiovascular (CV) risk factors. Overall, 44(45%), 18(18%), and 21(21%) patients achieved remission based on 28-joint disease activity score (DAS28), Boolean, and simplified disease activity score (SDAI) criteria at 12 months, respectively. Patients with detectable IL-33 at baseline were less likely to achieve DAS28 ($P = 0.010$) and SDAI remission ($P = 0.021$), while a lower baseline sST2 level was able to predict DAS28, Boolean, and SDAI remission ($P = 0.005$, 0.001, and < 0.001, respectively). Using multivariate analysis, a lower baseline sST2 level independently predict Boolean (OR = 0.789; $P = 0.005$) and SDAI remission (0.812; $P = 0.008$). Regarding carotid atherosclerosis, 9/98(9.2%) patients had plaque progression at 12 months. Baseline IL-33 was detectable in 8/9(89%) and 42/83(51%) of patients with and without plaque progression respectively ($P = 0.029$). Baseline detectable IL-33 was an independent predictor for plaque progression after adjusting for traditional CV risk factors ($P = 0.017$).
Conclusions: Lower baseline sST2 levels independently predict disease remission and baseline detectable IL-33 independently predicts carotid plaque progression in ERA patients. This study suggests that inflammation induced by the IL-33/ST2 axis may play a significant role in the development of cardiovascular disease in RA.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by persistent intense immunologic activity, local destruction of joints, and a variety of systemic manifestations [1]. The mainstay of treatment for RA includes synthetic and biological disease-modifying antirheumatic drugs (DMARDs) [2]. For early RA (ERA) patients, clinical remission is the recommended therapeutic goal [2]. However, a large proportion of patients failed to achieve remission even when combined therapy of synthetic and biological DMARDs was administrated [3]. Identification of

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predictors for response to treatment may not only provide risk estimation, but also help the development of personalized medicine and new therapeutic targets [4].

RA patients die prematurely compared with the general population [5], primarily because of cardiovascular diseases (CVD) [5,6]. Recent evidence suggested that this phenomenon also occurs in patients with ERA [7–9]. It has been hypothesized that upregulated proinflammatory cytokines/chemokines in RA patients accelerates atherogenesis leading to CVD [10,11]. Chronic inflammatory burden, reflected by the mean C-reactive protein (CRP) levels, were associated with increased carotid intima-media thickness (IMT) and augmented the risk of CV events and mortalities in established RA patients [12,13]. Nonetheless, cumulative average of CRP failed to establish a robust association with atheromatous plaque formation in other studies [14,15]. These findings indicate that there may be unidentified inflammatory pathways that mediate atherogenesis in this population.

Interleukin-33 (IL-33) is a recently discovered member of the IL-1 cytokine family [16]. Similar to IL-1α, IL-33 displays both nuclear and extracellular effects [17,18]. It could be released upon cell injury and served as an alarmin, and act extracellularly as a ligand for the IL-1 receptor family member ST2. The binding of IL-33 to ST2 further activates the nuclear factor-κB (NFκB) and mitogen-activated protein kinases (MAPK) pathways, leading to increased transcription of Th2 cytokines [16]. The ST2 receptor has two isoforms, a soluble (sST2) and a membrane bound form (ST2L), which are produced through differential mRNA processing. sST2 is an alternative spliced product of ST2, which lacks the transmembrane and intercellular domains. Thus, sST2 acts as a decoy receptor for IL-33 and inhibits IL-33/ST2 signaling [19]. IL-33/ST2 pathway is involved in the pathogenesis of RA. Experimental animal models revealed that administration of IL-33 exacerbates collagen-induced arthritis (CIA) [20], while blocking ST2 attenuate CIA [20,21]. In RA patients, IL-33 is significantly elevated both in synovial fluid and serum [22–25]. Serum levels of IL-33 significantly decrease after treatment [25,26]. Interestingly, serum sST2 levels also elevate in RA patients and decrease after treatment [24,25]. On the other hand, IL-33 can reduce atherosclerosis development in atherosclerotic mice on a high-fat diet through induction of Th2-mediated immunity and generation of protective autoantibodies [27]. This protective effect could be blocked by sST2 [27]. However, such effect has not been observed in human patients. Whether baseline IL-33 and sST2 levels in patients with RA are associated with response to treatment or development of atherosclerosis has never been examined. Prospective studies are of interest to determine the utility of IL-33 and sST2 as prognostic biomarkers of treatment response and vascular damage.

The objectives of the study were to elucidate the association between the baseline IL-33 and sST2 levels and (1) disease remission and (2) progression of carotid atherosclerosis in a cohort of ERA patients.

Materials and methods

Study population

Inclusion and exclusion criteria

Participants were enrolled in the Early Rheumatoid Arthritis Vascular Study, a prospective cohort study investigating subclinical CVD in ERA, which has been described in detail previously [28,29]. Briefly, patients who were age 18 or older and fulfilled the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria of RA [30], who had a symptom onset within 2 years from the date of assessment, were recruited. Patients were ineligible if they had a history of overt CVD defined as coronary heart disease, cerebrovascular event, peripheral arterial disease, or heart failure.

Study protocol

A total of 98 consecutive ERA patients followed up at the Rheumatology clinic of the Prince of Wales Hospital were recruited for this prospective study. Patients were examined clinically at baseline and every 3 months. Carotid ultrasound was performed at baseline and 12 months. Disease remission at 12 months was ascertained in all patients according to the following definitions: 28-joint disease activity score (DAS28) <2.6 [31] and 2011 ACR/EULAR definition [Boolean based: Tender Joint Count $\leq$ 1, Swollen Joint Count $\leq$ 1, CRP $\leq$ 1 mg/dL, Patient Global Assessment $\leq$ 1 (on a 0–10 numeric rating scale, NRS); Index based: simplified disease activity score (SDAI) $\leq$ 3.3] [32]. Ethics approval was obtained from Ethics Committee of The Chinese University of Hong Kong–New Territories East Cluster Hospitals, and written informed consent was obtained from all participants according to the Declaration of Helsinki.

Clinical interview

At baseline, we quantified the extent of disease by recording extra-articular manifestations, active joint count, number of damaged joints [33], and the patient's score on the modified health assessment questionnaire (HAQ) [34]. Previous treatments were recorded by patient interview and chart review. Other data including smoking habits, medical history, and drug treatment were noted. Anthropomorphic measurements were also recorded. All patients were interviewed and examined using standardized data collection instruments.

Treatment protocol

Patients were assessed by rheumatologists (LST, WPK, or EKL) every 3 months. A total of 40 patients with active disease [$\geq$4 swollen and tender joints, DAS28 $>$ 3.2, and erythrocyte sedimentation rate (ESR) $\geq$ 28 mm/h or CRP $\geq$ 10 mg/L] were recruited for a randomized trial on methotrexate (MTX) with or without infliximab to ascertain the efficacy of these drugs on the changes in vascular assessments; details have been published elsewhere [29]. For the remaining 58 patients with mild to moderate disease activity who did not fulfill the criteria for the randomized trial, adjustment of treatment was made according to a second protocol if patients could not achieve DAS28 remission every 3 months (Supplementary Figure) [28]. Briefly, MTX would be started as monotherapy; other synthetic DMARDs could be used if a contraindication for MTX was present. Combination synthetic or biologic DMARDs would be given to patients who failed to respond to monotherapy. If clinical remission was consistently achieved for at least 6 months, medication was gradually tapered until 1 drug remained at a maintenance dose [28].

Laboratory tests

Complete blood count, liver and renal function tests, ESR and CRP, fasting blood glucose, and lipid profile [total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides] were checked every 3 months.

Quantitative analysis of IL-33, sST2, and inflammatory cytokines

We collected 10 mL of ethylenediaminetetraacetic acid (EDTA) blood from patients. The EDTA blood samples were centrifuged at 2000*g* for 15 min to obtain plasma. Plasma samples were stored at -70°C immediately until analysis.