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Plasma interleukin-38 in patients with rheumatoid arthritis

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ABSTRACT

Previous studies have indicated that interleukin-38 (IL-38) is involved in rheumatoid arthritis (RA). The present study aims to assess plasma levels of IL-38 in RA and discuss the potential of IL-38 as a biomarker for RA. Protein concentrations of IL-38 were examined by enzyme-linked immunosorbent assay, and the mRNA level of IL-38 was tested by quantitative real-time polymerase chain reaction. Plasma IL-38 was first compared in a training cohort, including 130 RA patients and 53 healthy controls, given the optimal cutoff. Then, we validated the levels of IL-38 in a further cohort of 519 patients, including 250 with RA, 63 systemic lupus erythematosus, 62 primary Sjogren's syndrome, 51 gout, 63 osteoarthritis, and 30 psoriatic arthritis, as well as 60 healthy controls. To further discuss the changes in IL-38 after treatment and the relationship with disease activity, we tested IL-38 expression in RA patients from the training cohort under follow-up. In the training cohort, plasma levels of IL-38 were higher in RA patients compared with healthy controls (681.00 [234.45–826.47] versus 152.04 [70.06–246.80] pg/mL, $P < 0.001$). The IL-38 mRNA level was elevated in RA patients as compared with healthy controls ($P < 0.001$). Expression of IL-38 was significantly higher in RA patients compared with that in non-RA patients in the validation cohort (all $P < 0.001$). Treatment significantly reduced IL-38 expression. IL-38 expression was related to parameters of inflammation both at baseline and in the follow-up studies. The area under the curve (AUC) of the receiver-operating characteristic curve showed that IL-38 may be a potential biomarker for RA. At the optimal cutoff value of 341.90 pg/mL, the sensitivity, specificity, and AUC were 72.30%, 90.60%, and 0.840, respectively, in the training cohort. Similar results were noted in the validation cohort. In conclusion, IL-38 expression correlated with RA disease activity, and plasma IL-38 might be a promising diagnostic biomarker for RA.

1. Introduction

The cytokines of the interleukin (IL)-1 family are a heterogeneous group of proteins that exhibit a broad spectrum of immunity functions. This family contains 7 agonists (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ), and 4 members display antagonist or anti-inflammatory activity (IL-1 receptor antagonist [IL-1Ra], IL-36Ra, IL-37, and IL-38).

IL-38 was cloned and characterized as a member of the IL-1 family of ligands in 2001 [1,2]. The human IL-38 gene is located on chromosome 2q13-14.1 [3]. IL-38 is expressed in a variety of tissues such as heart, placenta, spleen, and thymus. IL-38 shares 41% and 43% homology with IL-1Ra and IL-36Ra, respectively [2]. IL-38 lacks a signal peptide and caspase-1 consensus cleavage site [1,4]. Nevertheless, the N terminus for premature IL-38 is still unclear [4]. IL-38 also shows a 12-β-stranded trefoil structure and shares the crystal structure of IL-1Ra and IL-18 [1,5]. IL-38 can bind to IL-36R and inhibit the proinflammatory function of the three agonist ligands IL-36α, IL-36β, and IL-36γ [6,7].

Genetic studies indicated a correlation between IL-38 and inflammatory diseases, such as spondyloarthritis, rheumatoid arthritis (RA), and psoriatic arthritis (PsA) [8]. A genome-wide association study reported IL-38 as 1 of 18 markers related to increased C-reactive protein (CRP) levels [9]. IL-38 expression was increased in minor labial salivary glands of primary Sjogren's syndrome (pSS) patients [10] and elevated in serum of patients with pustular psoriasis compared with healthy controls [11]. However, peripheral blood mononuclear cells (PBMCs) from healthy volunteers exposed to *Candida albicans* showed a clear Th17 response, showing a significant production of IL-17A and IL-22, and the presence of IL-38 reduced the production of IL-17A and IL-22

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by 37% and 39%, respectively [6]. IL-38 deficiency in apoptotic cells up-regulated IL-6 and IL-8 expression and IL-38 knockdown in human primary macrophages activated by AP-1 [12]. PBMCs from healthy volunteers transfected with IL-38 siRNA produced more proinflammatory components of IL-6, CCL2, and APRIL than did control siRNA-transfected cells [13]. These findings imply that there may be a negative feedback mechanism for IL-38 and that IL-38 inhibits excessive inflammation.

RA is an inflammatory autoimmune disease. Aberrant release of cytokines has been reported in RA patients and animal models. Although the expression levels of IL-38 in the synovium of RA patients and joints of mice with arthritis were found to be elevated [14–16], its relationship with disease activity and its clinical association and disease manifestations in RA are still unclear. In this study, we performed a multistage design to determine the plasma levels of IL-38 in RA patients in a training and validation cohort and evaluated the potential of IL-38 as a biomarker for RA.

2. Materials and methods

2.1. Patients

Between July 2017 and May 2018, 380 patients older than 18 years who fulfilled the 1987 American College of Rheumatology revised criteria for RA [17] participated in a longitudinal study of disease activity and biomarkers. The patients received care at the Department of Rheumatology and Immunology, Affiliated Hospital of Southwest Medical University and Affiliated Minda Hospital of Hubei Institute for Nationalities. The present study aims to identify whether plasma IL-38 can be used to distinguish RA patients from healthy controls and different rheumatic autoimmune disease patients. In addition, we discussed the changes in plasma IL-38 in RA patients after 6 months of treatment. This was performed using a training cohort including 130 RA patients and 53 healthy volunteers (Table 1, Supplementary Table 1). Next, we validated the diagnostic value of plasma IL-38 in RA with a larger cohort including 519 patients and 60 healthy controls, which included 250 RA, 63 systemic lupus erythematosus (SLE), 62 pSS, 51 gout, 63 osteoarthritis (OA), and 30 PsA patients (Supplementary Table 2). In addition, RA patients in the training set were followed up twice every 3 months so as to discuss the changes in IL-38. Birth date, sex, body mass index, and year of disease onset were recorded upon admission. Demographic, clinical, and laboratory data were collected from hospital records or by questionnaire under follow-up. RA patients and healthy controls in the training cohort and validation cohort were matched by age and sex. The disease severity of each RA patient was assessed by disease activity score 28 (DAS28): high (DAS28 > 5.1), moderate (3.2 < DAS28 < 5.1), low disease activity (2.6 < DAS28 < 3.2), and remission (DAS28 < 2.6) [18,19]. Among RA patients in the training cohort, 115 had an initial DAS28 score > 3.2. Stratifying by disease duration, patients with a disease duration of < 18 months were considered to have early RA, and patients with a disease duration > 18 months were recognized as having longer RA. Before treatment, blood samples were collected from all patients. RA patients in the training cohort completed clinical data and plasma samples from the follow-up within half a year.

The study was approved by the Medical Ethics Committee of Southwest Medical University. Methods were carried out in accordance with the approved guidelines. All subjects were enrolled after informed consent had been obtained.

2.2. Laboratory tests

The erythrocyte sedimentation rates (ESRs) of each patient were examined. Levels of serum CRP, rheumatoid factor (RF), and anti-cyclic citrullinated peptide (anti-CCP) were examined by scatter turbidimetry (Siemens Healthcare Diagnostics Products, GmbH, Germany). Serum levels of RF higher than 40 IU/mL were recognized as RF positive, and serum concentrations of CRP higher than 8 mg/L were considered as positive. Antinuclear antibody was measured by line blot techniques (Euroimmun, Lubeck, Germany).

Table 1
Characteristics of RA patients and healthy controls in the training cohort (at baseline).

Characteristics	RA patients (N = 130)	Healthy controls (N = 53)	*P* value
Age (year)	52.3 ± 11.88	51.4 ± 12.06	0.799
Sex (male/female)	15/115	6/47	0.608
BMI (kg/m^2)	22.10 ± 2.58	–	–
Disease duration (years)	3.01 (1.29–10.26)	–	–
ANA (n, %)	16 (12.3)	–	–
Anti-CCP (n, %)	85 (65.4)	–	–
RF (n, %)	102 (78.5)	–	–
ESR (mm/H)	33.15 (20.70–46.85)	–	–
CRP (mg/L)	7.59 (3.26–15.65)	–	–
DAS28 (ESR)	4.27 (3.53–5.26)	–	–
DAS28 (CRP)	3.74 (2.99–4.65)	–	–
HAQ	0.25 (0.09–0.81)	–	–

BMI, body mass index; ANA, antinuclear antibody; Anti-CCP, anti-cyclic citrullinated peptide; ESRs, erythrocyte sedimentation rates; RF, rheumatoid factor; CRP, C-reactive protein; DAS28, disease activity score 28; HAQ, health assessment questionnaire; RA, rheumatoid arthritis.

2.3. IL-38 cytokine quantification

Venous blood was collected during laboratory test. Plasma was obtained and stored at −80 °C until use, as described [20,21]. Concentrations of IL-38 were measured using specific enzyme-linked immunosorbent assays (ELISAs; AdipoGen, Liestal, Switzerland). The results of IL-38 were expressed as picograms per milliliter (pg/mL). The inter-assay and intra-assay reliability of the IL-38 assays were 5.5% and 4.5%, respectively, and the lower detection limit of the assay was 31 pg/mL. Each sample was tested in duplicate.

2.4. RNA extraction and real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from PBMCs using TRIzol following the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA) and quantified using a NanoDrop (ND-1000) spectrophotometer (Thermo Scientific, Hudson, NH, USA). cDNAs were prepared via the iScript™ cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA). PCR primers (Generay, Shanghai, China) were as follows: IL-38, sense: 5′-CCTCCCCATGGCA AGATACT-3′ and 5′-GCCAAGCCTCTGTTAGGAAG-3′ (reverse); β-actin, sense: 5′-CTCCCTGGAGAAGAGCTACGAGC-3′ and 5′-CCAGGAAGGAA GGCTGGAAGAG-3′ (reverse). Each RT-PCR was prepared in a 20 μL reaction mixture containing 10 μL SYBR Green PCR Master Mix, 1 μL cDNA, and 1.4 μL primers conducted on an ABI Prism 7900 sequence detector (Applied Biosystems, Carlsbad, CA, USA). All samples of RA patients and controls were assayed in triplicate. Relative gene expression was determined by the $2^{-\Delta\Delta ct}$ method.

2.5. Statistical analysis

Data were discriminated by normality, by which normal data were expressed as mean ± SD, whereas the median (interquartile range) was used to express the nonnormal data. Comparison of quantitative values was performed by Mann-Whitney *U* test for unpaired data or Student's *t*-test. The area under the curve (AUC) of the receiver-operating characteristic (ROC) curves evaluated the potential of IL-38, RF, and CRP as biomarkers for RA. Statistical analyses were performed by SPSS 10.01 (SPSS Inc., Chicago, IL, USA). The ROC curve analyses were performed with MedCalc 11.4.2.0 (Mariakerke, Belgium). A *P*