The Egyptian Rheumatologist (2016) 38, 99–104

HOSTED BY

Egyptian Society of Rheumatic Diseases

The Egyptian Rheumatologist

www.rheumatology.eg.net
www.elsevier.com/locate/ejr

ORIGINAL ARTICLE

# Serum level of interleukin-33 in rheumatoid arthritis patients and its association with bone erosion and interstitial lung disease

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Received 27 April 2015; accepted 5 June 2015
Available online 25 April 2016

**KEYWORDS**

Rheumatoid arthritis;
IL-33;
DAS-28;
Bone erosions;
ILD

**Abstract** *Aim of the work:* To analyze the serum levels of IL-33 in RA patients and to investigate its relation to the clinical characteristics, laboratory investigations, joint erosions, functional status and disease activity. Its relation to the presence of interstitial lung disease (ILD) was well thought-out.

*Patients and methods:* The study included 50 RA patients and 30 matched control. Thorough clinical examination, investigations, disease activity score (DAS-28) and health assessment questionnaire (HAQ) were considered in the patients. Bone erosion was evaluated and interstitial lung disease (ILD) was identified on high-resolution computed tomography. The serum level of IL-33 was measured by enzyme-linked immunosorbent assay.

*Results:* Serum levels of IL-33 are significantly higher in RA patients (106.96 ± 52.6 pg/ml) than in healthy controls (46.9 ± 23 pg/ml) ($p < 0.001$). A significant correlation was found between IL-33 and the DAS28 ($r = 0.4$, $p = 0.001$), level of rheumatoid factor ($r = 0.45$, $p = 0.001$) and with the presence of ILD ($r = 0.3$, $p = 0.04$). There were no gender differences and the level did not significantly correlate with the age or disease duration. The medications received had no obvious effect on the IL-33 level. The level did not correlate with the HAQ. There was a significant correlation between the CT bone erosion scores the patient's age, disease duration, rheumatoid nodules and DAS28. The erosion score also significantly correlated with the serum IL-33 levels in RA patients ($r = 0.71$, $p = 0.001$).

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Peer review under responsibility of Egyptian Society of Rheumatic Diseases.

http://dx.doi.org/10.1016/j.ejr.2015.06.001

*Conclusion:* These data support the hypothesis that IL-33 may be involved in RA pathogenesis and it may partly contribute to the bone erosion and ILD in RA patients.



## 1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammatory response, including synovial proliferation and excessive proinflammatory cytokine production, leading to eventual cartilage and bone destruction [1]. Several mediators and factors were reported to play a role in Egyptian RA patients including oxidative stress [2–4], T-regulatory cells (Treg) and the imbalance of the Treg/TH17 cytokine axis [5] and cytokines [6,7] have also been implicated in the pathogenic mechanisms of RA. Several proinflammatory cytokines are considered critical in forming the inflammatory process of RA [1]. There has been much evidence confirming the involvement of IL-33 in RA.

Interleukin-33 (IL-33) is a newly reported cytokine of IL-1 family, which has been demonstrated to induce cytokine syntheses and mediate inflammatory responses through its receptor ST2. It is widely expressed in many tissues such as the liver, lung, central nervous system and multiple types of cells including epithelial, endothelial, smooth muscle, macrophages and fibroblasts [8]. Moreover, it is mainly localized in the nucleus but under appropriate signal stimulation such as inflammation, IL-33 is processed and passively released from necrotic cells or actively secreted into the extracellular milieu [9]. Through binding to its receptor ST2, it functions as a proinflammatory cytokine that participates in the development and progression of many diseases including collagen-induced arthritis (CIA) [10], inflammatory bowel disease [11], autoimmune hepatitis, anaphylactic shock [12] and ischemia reperfusion injury [13,14].

It has been reported that administration of sST2 fusion protein dramatically attenuated disease severity by reducing cellular infiltration in the joints, synovial hyperplasia and joint erosion due to inhibiting the release of proinflammatory cytokines comprising IL-6, IL-12, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). As high expression levels of IL-33 in human RA synovium have been discovered, treatment with an ST2 blocking antibody at disease attenuated the severity of CIA and reduced joint destruction. This highly suggested a critical contribution of locally produced IL-33 to the pathogenesis of joint inflammation and destruction [10].

Interstitial lung disease is a dreaded complication of RA. The most common pattern on high resolution computerized tomography (HRCT) and histopathology is usual interstitial pneumonia (UIP), with nonspecific interstitial pneumonia seen less frequently. Pulmonary function testing most commonly shows reduced diffusion capacity for carbon monoxide and HRCT reveals a combination of reticulation and ground glass abnormalities [15]. Three dimensions computed tomography (3D-CT) is a tomographic imaging method offering high resolution, especially of cortical bone and three dimensional visualization of calcified tissue, allowing clear definition of the margins of erosion. The 3D-CT can provide a clear impression of lesion extent, pattern, shape and proximity to adjacent structures [16].

The aim of the present work was to analyze the serum levels of IL-33 in RA patients and to investigate its relation to the clinical characteristics, laboratory investigations, joint erosions, functional status and disease activity. Its relation to the presence of ILD was well thought-out.

## 2. Patients and methods

This study was carried out on 50 RA cases diagnosed according to ACR criteria of rheumatoid arthritis [17] were selected from those attending the outpatient Rheumatology clinic of Zagazig University. The patients were 9 males and 41 females, their age ranged from 34 to 69 years with a mean age of 51.1 ± 9.6 years and the duration of the disease ranged from 5 to 23 years with a mean of 11.4 ± 4.9 years. Twenty age and sex matched healthy controls were included. Patients with liver disease, history of anaphylactic shock or ischemia and any other rheumatic disease were excluded. The study was approved by the local ethics committee and consent from the patients was taken before being enrolled in the study.

Full history was taken from the patients, clinical examination performed, disease activity score in 28 joints (DAS28) calculated [18] and function status estimated using the health assessment questionnaire [19]. Laboratory investigations were performed including complete blood count (CBC), Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and Rheumatoid factor (RF). Interleukin-33 (IL-33) was assessed by Enzyme-Linked Immunosorbent Assay (ELISA) in both patients and control.

### 2.1. Quantitative measurement of IL-33

Human IL-33 PicoKine™ ELISA Kit was used. The assay employs an antibody specific for human IL-33 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-33 present in a sample is bound by the immobilized antibody. The wells are washed and biotinylated anti-human IL-33 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added and color develops in proportion to the amount of IL-33 bound. The Stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. We calculated the mean absorbance for each set of duplicate standards, controls and samples, and subtracted the average zero standard optical density. The standard curve was plotted on log–log graph paper or by using Sigma plot