# Interleukin-33 as a marker for disease activity in rheumatoid arthritis

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**Background**
Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder thought to be autoimmune in nature and predominately affects synovial joints. Interleukin-33 (IL-33) is a newly reported cytokine of the IL-1 family.
**Aim of the work**
The aim of this study was to assess the role of IL-33 in the pathogenesis of RA.
**Patients and methods**
Group A included 30 adult patients with RA; all cases were diagnosed according to the American College of Rheumatology criteria for RA. Group B included 20 healthy adult persons (age and sex matched) who comprised the control group. The serum IL-33 levels were examined by using the enzyme-linked immunosorbent assay for 30 patients with RA and 20 healthy individuals. Disease activity was assessed according to disease activity score 28–C-reactive protein (CRP) scale.
**Results**
IL-33 was increased in all RA patients compared with controls. IL-33 was highly correlated to erythrocyte sedimentation rate, CRP, rheumatoid factor, anti-cyclic citrullinated peptide, and disease activity score 28–CRP score. Therefore, IL-33 most probably has a significant role to play in the pathogenesis of RA.
**Conclusion**
IL-33 most probably has a significant role in the pathogenesis of RA. IL-33 serum levels paralleled the severity of the disease subset. Understanding the functions of IL-33 is important for the development of new therapeutic approaches including IL-33 inhibitors as a therapeutic target.

**Keywords:**
IL-33 interleukin -33, disease activity score, rheumatoid arthritis

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## Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder thought to be autoimmune in nature and predominately affects the synovial joints. Inflammation of the synovium is associated with hyperplasia of synovial cells, excess synovial fluid, and pannus formation. Pannus represents a thickened membrane-like covering of inflammatory granulation tissue over the articular cartilage [1]. The clinical hallmark of RA is polyarticular synovial inflammation of peripheral joints – typically in the hands [meta-carpophalangeal (MCP) joints and proximal inter-phalangeal joints], causing pain, stiffness, and often some degree of irreversible joint damage, deformity, and disability. In addition, there is also a significant systemic inflammatory state present that may promote a number of other extra-articular effects including coronary artery disease, pulmonary fibrosis, osteoporosis, and vasculitis [2]. Although the etiology of RA is unknown, many studies suggest that a combination of environmental and genetic factors is responsible [3].

### Epidemiology

RA is one of many autoimmune diseases that is predominant in women, with a female to male ratio ranging from 2: 1 to 3: 1[4].

### Etiology of rheumatoid arthritis

#### *Tobacco*

Smoking is the best defined environmental risk factor for seropositive RA in certain populations.[3]

#### *Infectious cause*

Although genetic factors predispose an individual to developing RA, the environment [5] clearly contributes. The pathogen potentially could initiate the disease through a variety of mechanisms, including direct infection of the synovium, activation of innate

immunity by pattern-recognition receptors that bind to the components of the agent, or through molecular mimicry that induces an autoreactive adaptive immune response.

#### Pathophysiology

An external trigger (cigarette smoking, infection, or trauma)[3,6] that initiates an autoimmune reaction, leading to synovial hypertrophy and chronic joint inflammation along with the potential for extra-articular manifestations, is theorized to occur in genetically susceptible individuals. CD4 T-cells, mononuclear phagocytes, fibroblasts, osteoclasts, and neutrophils play major cellular roles in the pathophysiology of RA, whereas B-cells produce autoantibodies [i.e. rheumatoid factors (RFs)]. Abnormal production of numerous cytokines, chemokines, and other inflammatory mediators[7] [e.g. tumor necrosis factor α (TNF-α), interleukin (IL)-1, IL-6, IL-8, transforming growth factor β, fibroblast growth factor, and platelet-derived growth factor] has been demonstrated in patients with RA.

#### Pathogenesis

Many proinflammatory cytokines play a key role in the processes of RA, such as TNF-α, IL-1, and IL-6 [8].

They play an integral role in the initiation and perpetuation of synovitis. Factors produced by T-lymphocytes are low in RA, whereas factors generated by macrophages and by synovial fibroblasts are markedly increased [9].

The IL-1 [10] family is a ubiquitous group of polypeptides with a wide range of biologic activity; they include IL-1α, IL-1β, IL-18, and IL-1Ra (a natural inhibitor of IL-1). Abundant animal data indicate that IL-1 can serve as a key regulatory factor in inflammatory arthritis. IL-1 has been implicated in RA, and inhibition of this mediator using IL-1Ra (anakinra) [11] has modest anti-inflammatory activities in humans.

#### Clinical picture of rheumatoid arthritis

The joints most commonly involved first in RA are the MCP joints, proximal interphalangeal joints, MCP joints, and wrists [12].

#### Rheumatoid nodule [13]

Nodules are found most often on extensor surfaces or pressure points.

Different types of deformities associated with RA include swan neck, boutonnière deformity, zigzag deformity, and trigger finger.

#### Hematological abnormalities

Hematological abnormalities include anemia, leukocytosis, and thrombocytosis [14].

#### Vasculitis

Distal arteritis, cutaneous ulceration, and peripheral neuropathy [15].

#### Heart and blood vessels

Pericarditis, endocarditis, left ventricular failure, valvulitis and fibrosis [16].

#### Interleukin-33

IL-33 is a protein that in humans is encoded by the IL-33 gene [17]. IL-33 is a member of the IL-1 family that potently drives the production of T-macrophages, and this induction is dependent on TNF-α and IL-1β [18]; IL-33 could induce and mediate neutrophil migration by activating synoviocytes and macrophages, and this induction is dependent on CXCL1, CCL3, TNF-α, and IL-1β.

## Patients

The present study included two groups. The protocol of this study was approved by the IRB/Ethics committee of the faculty of Medicine, Alexandria University.

Group A, which included 30 adult patients with RA; all these cases were diagnosed according to the American College of Rheumatology criteria for RA [19].

Group B, which included 20 healthy adult individuals (age and sex matched) who comprised the control group.

All patients were selected from Internal Medicine Department, Rheumatology Unit, Main Alexandria University Hospital.

An informed consent was taken from all patients before the their participation in the study.

## Methods

This study was conducted on 30 patients with RA and 20 healthy adults (the control group) admitted to the Rheumatology Unit of Alexandria Main University Hospital.

The study population was subjected to the following:

(1) Complete history taking including the following:
    (a) Demographic data.
    (b) History of the present complaints.

(2) Thorough clinical examination, which included the following:
Vital signs.
Head and neck examination.
Cardiovascular examination.
Chest examination.
Abdominal examination.
Skin and extremities' examination.
Joint examination.
Inspection of the joints was carried out as regards the presence of swelling, deformity, and signs of inflammation.
(i) Tenderness.
(ii) Presence of effusion.
(iii) Range of movement whether active or passive movement.
(iv) Presence of deformities.
(3) Disease activity in RA was assessed according to the disease activity score 28 (DAS28)–C-reactive protein (CRP) [20].
(4) Laboratory investigations included erythrocyte sedimentation rate (ESR), CRP, RF, and anti-cyclic citrullinated peptide (anti-CCP).

The serum level of IL-33 were determined by using the human enzyme-linked immunosorbent assay method.

## Results

Table 1 shows the comparison between the two studied groups regarding IL-33. In the present study, the level of IL-33 among the patients of RA ranged from 35 to 110 pg/ml with a mean of 77.23 ± 20.24.

Whereas in the control group, the level of the IL-33 ranged from 10 to 40 pg/ml with a mean of 19.3 ± 6.11.

**Table 1 Comparison between the two studied groups regarding IL-33**

| IL-33 | RA | Control group |
|---|---|---|
| Range | 35–110 | 10–40 |
| Mean | 77.23 | 19.3 |
| SD | 20.24 | 6.11 |
| *t* | 9.85 | |
| *P* | 0.001 | |

IL-33, interleukin-33; RA, rheumatoid arthritis.

Values higher than the mean ± 2 SD (31.52 pg/ml) of the control serum sample were considered to be elevated in this study. The level of IL-33 among the patients of RA were higher in comparison with the control group, and this difference was statistically significant ($P=0.001$) (Fig. 1).

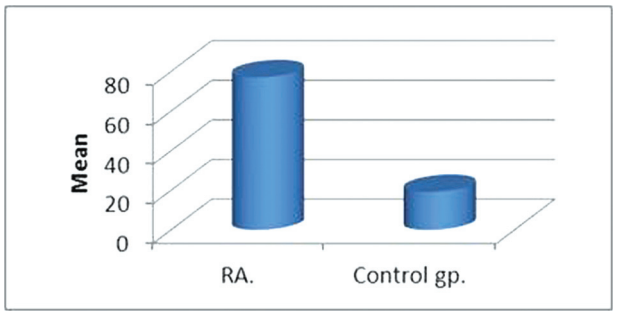
Table 2 shows the comparison between IL-33 and DAS28–CRP scores for the RA patients. There was a significant positive relation between IL-33 and DAS28–CRP scores ($P<0.05$); the elevated level of IL-33 in RA patients was correlated with severe disease activity compared with the moderate or low-activity group (Fig. 2).

Table 3 shows the correlation between IL-33 and different disease activity parameters. The level of DAS28–CRP among the patients of RA ranged from 2.7 to 5.6 with a mean of 4.30 ± 0.85; ESR ranged from 20 to 140 with a mean of 74.37 ± 30.12, CRP ranged from 8 to 80 with a mean of 28.10 ± 15.25, RF ranged from 24 to 49 with a mean of 38.37 ± 7.18, and anti-CCP ranged from 25 to 398 with a mean 85.67 ± 74.22. There were significant positive correlations between IL-33 and different disease activity parameters (DAS28–CRP, ESR, CRP, RF, and anti-CCP) ($P<0.05$) (Figs. 3–7).

## Discussion

The present study was conducted on 30 patients with RA diagnosed according to the American College of

**Figure 1**




Comparison between the two studied groups regarding IL-33. IL-33, interleukin-33; RA, rheumatoid arthritis.

**Table 2 Comparison between IL-33 and DAS28–CRP score in RA patients**

| Activity | DAS28–CRP score | | IL-33 level | |
|---|---|---|---|---|
| | Minimum–maximum | Mean ± SD | Minimum–maximum | Mean ± SD |
| Mild | 2.7–3.1 | 2.84 ± 0.15 | 35.0–75.5 | 52.6 ± 6.98 |
| Moderate | 3.4–5 | 4.40 ± 0.42 | 42.0–85.0 | 64.5 ± 11.02 |
| Severe | 5.3–5.6 | 5.48 ± 0.11 | 77.3–110 | 85.2 ± 8.98 |
| *r* | 0.39 | | | |
| *P* | 0.025 | | | |

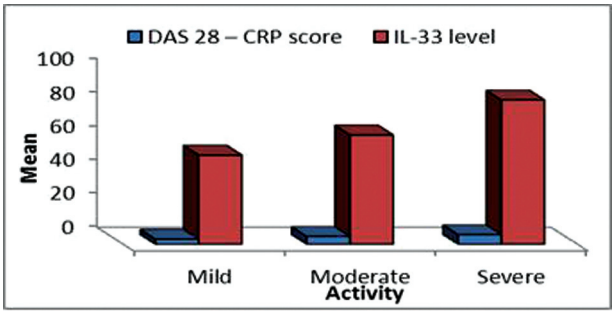
DAS28–CRP, disease activity score 28–C-reactive protein; IL-33, interleukin-33; RA, rheumatoid arthritis.

**Table 3 Correlation between IL-33 and different disease activity parameters**

| Disease activity parameters | Range | Mean ± SD | IL-33 | |
|---|---|---|---|---|
| | | | *r* | *P* |
| DAS28–CRP | 2.7–5.6 | 4.30 ± 0.85 | 0.39 | 0.025 |
| ESR | 20–140 | 74.37 ± 30.12 | 0.227 | 0.023 |
| CRP | 8–80 | 28.10 ± 15.25 | 0.365 | 0.039 |
| RF | 24–49 | 38.37 ± 7.18 | 0.400 | 0.0291 |
| Anti-CCP | 25–398 | 85.67 ± 74.22 | 0.463 | 0.021 |

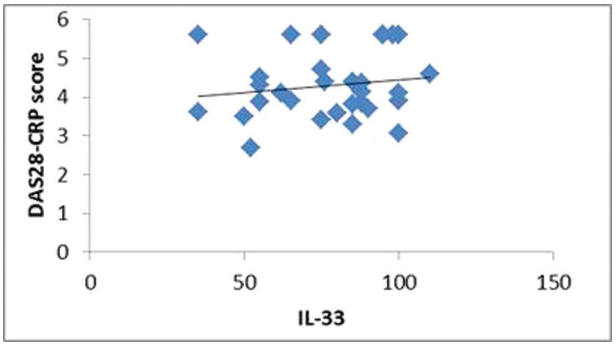
CCP, cyclic citrullinated peptide; DAS28–CRP, disease activity score 28–C-reactive protein; ESR, erythrocyte sedimentation rate; IL-33, interleukin-33; RF, rheumatoid factor.

**Figure 2**



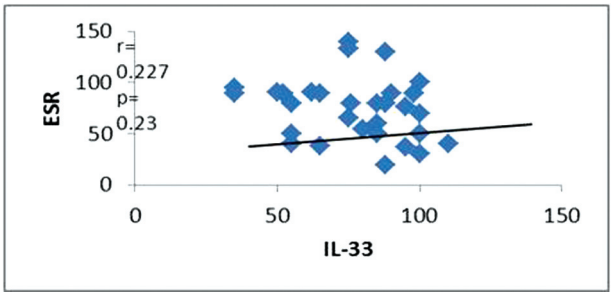

Comparison between IL-33 and DAS28–CRP score in RA patients. DAS28–CRP, disease activity score 28–C-reactive protein; IL-33, interleukin-33; RA, rheumatoid arthritis.

**Figure 3**



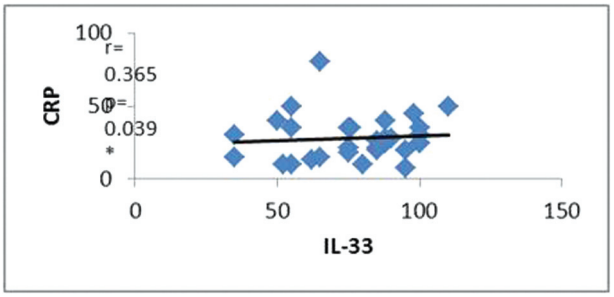

Correlation between IL-33 and DAS28–CRP score. DAS28–CRP, disease activity score 28–C-reactive protein; IL-33, interleukin-33.

**Figure 4**



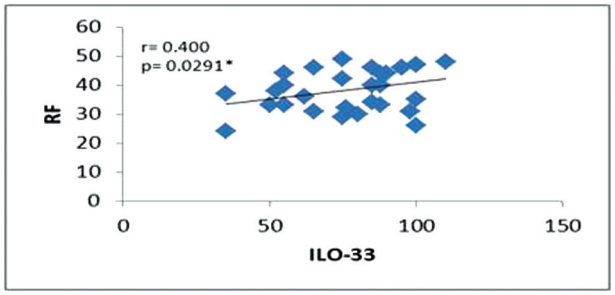

Correlation between IL-33 and ESR. ESR, erythrocyte sedimentation rate; IL-33, interleukin-33.

**Figure 5**



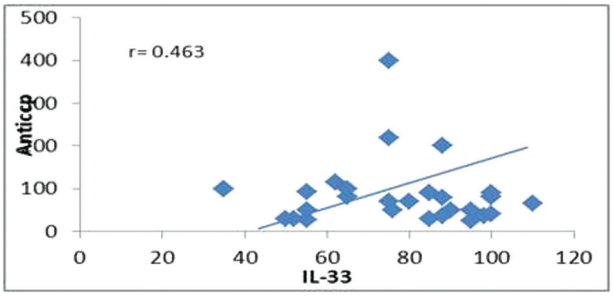

Correlation between IL-33 and CRP. CRP, C-reactive protein; IL-33, interleukin-33.

**Figure 6**




Correlation between IL-33 and RF. RF, rheumatoid factor; IL-33, interleukin-33.

**Figure 7**




Correlation between IL-33 and anti-CCP. CCP, cyclic citrullinated peptide; IL-33, interleukin-33.

Rheumatology criteria [19] and 20 age-matched and sex-matched healthy adults (the control group).

The level of IL-33 among the patients of RA ranged from 35 to 110 pg/ml with a mean of 77.23 ± 20.24.

Whereas, in the control group, the level of the IL-33 ranged from 10 to 40 pg/ml with a mean of 19.3 ± 6.11.

The level of IL-33 among the patients of RA was higher compared with the control group, and this difference was of statistical significance ($P = 0.001$).

Similar results have been reported in several studies including that of Hong *et al.*[21]; they reported that in patients with RA, the serum levels of IL-33 and sST2 were significantly higher than that of healthy controls. Furthermore, Xiangyang *et al.*[22] reported a positive correlation between the levels of IL-33 and RF, thus supporting the idea that IL-33 is implicated in the pathogenesis of RA. As in our study, there was a positive correlation between IL-33 and ESR, CRP, RF, anti-CCP, and DAS28–CRP. Similar results were reported by Matsuyama *et al.*[23], who demonstrated that DAS28 scores based on CRP levels (DAS28–CRP) were significantly higher in patients with detectable levels of IL-33 in sera than in those with undetectable levels. In a study conducted by Palmer *et al.*[24] it was found that there were high expression levels of IL-33 in human RA synovium and experimental arthritis. Moreover, treatment with an ST2 blocking antibody at the onset of disease reduced joint destruction, which clearly suggested that locally produced IL-33 may contribute to the pathogenesis of joint inflammation and destruction. In a study by Kageyama *et al.*[25], to determine the relationship between IL-33 and TNF-α in RA pathogenesis. For RA patients, by administrating etanercept (a TNF-α inhibitor), the serum level of IL-33 significantly decreased at 3 and 6 months, and serum IL-33 levels showed a significant correlation with the number of tender joints, CRP, DAS of 28 joints including CRP and the white blood cells count, and an inverse correlation with the red blood cells count and the hemoglobin level.

Thus, the findings of our study suggest that IL-33 most probably plays a significant role in the pathogenesis of RA and contributes to the bone erosion in RA patients.

## Conclusion

The following conclusions can be drawn from the current study.

As IL-33 is highly expressed in the serum of RA patients, we can conclude that IL-33 most probably has a significant role in the pathogenesis of RA.

IL-33 is highly correlated to ESR, CRP, RF, anti-CCP, and DAS28–CRP score.

Therefore, understanding the functions of IL-33 is important for the development of new therapeutic approaches including IL-33 inhibitors as a therapeutic target.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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