Interleukin-34 in the Serum and Synovial Fluid of Rheumatoid Arthritis Patients: Relation to Disease Activity and Radiographic Damage

Interleukin-34 in Serum und Synovialflüssigkeit von Patienten mit rheumatoider Arthritis: Beziehung zu Krankheitsaktivität und radiologischer Schädigung

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Schlüsselwörter
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Bibliography
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ABSTRACT

Background
Interleukin-34 (IL-34) is a recently discovered proinflammatory cytokine that can promote inflammation and osteoclastogenesis in arthritic joints. In this study, we tried to assess the serum and synovial fluid (SF) levels of IL-34 in rheumatoid arthritis (RA) patients, and to determine its relationship with disease activity and radiographic damage.

Patients and methods
ELISA was used to evaluate IL-34 levels in the serum of RA patients (n = 50), osteoarthritis (OA) patients (n = 28), and healthy control subjects (n = 20) and in SF isolated from RA and OA patients. Disease activity in RA patients was assessed using the disease activity score-28 (DAS 28). The extent of radiographic joint damage, narrowing, and erosions was assessed.

Results
Serum IL-34 level was significantly elevated in RA patients compared to that in OA patients and healthy controls (P < 0.0001). Synovial IL-34 level was also significantly elevated in RA patients compared to that in OA patients (P = 0.0004). Serum and synovial IL-34 levels were significantly higher in cases that were rheumatoid factor (RF) positive (n = 28) compared to the levels in RF-negative RA cases (n = 22) (P = 0.03 and P = 0.04, respectively). Serum and synovial IL-34 levels were positively correlated with RF (r = 0.43, P = 0.02 and r = 0.39, P = 0.03, respectively), and with the extent of radiological damage (SENS) (P = 0.0002 and P < 0.0001, respectively). However, no significant correlation between IL-34 levels and disease activity was found.

Conclusion
IL-34 appears to play a key role in the pathogenesis of RA and contribute to bone destruction, making it a potential therapeutic target in the management armamentarium of the disease.

ZUSAMMENFASSUNG

Hintergrund
Interleukin 34 (IL-34) ist ein vor Kurzem entdecktes proinflammatorisches Zytokin, das die Entzündung und die Osteoklastogenese in arthritischen Gelenken begünstigen kann. In dieser Studie wurde versucht, die IL-34-Spiegel von Patienten mit rheumatoider Arthritis (RA) in Serum und Synovialflüssigkeit (SF) zu bestimmen und deren Beziehung zu Krankheitsaktivität und radiologischen Schäden zu ermitteln.

Patienten und Methoden
Mithilfe eines ELISA-Assays untersuchten wir die IL-34-Spiegel im Serum von RA-Patienten (n = 50), Patienten mit Arthrose (Osteoarthritis, OA) (n = 28) und gesunden Kontrollprobanden (n = 20) sowie in der von RA- und OA-Patienten isolierten SF. Mithilfe des Disease Activity
Score-28 (DAS 28) wurde die Krankheitsaktivität bei RA-Patienten bestimmt. Ferner wurde das Ausmaß radiologischer Gelenkschäden, Einengungen und Erosionen beurteilt.

**Ergebnisse** Der Serum-IL-34-Spiegel war bei den RA-Patienten im Vergleich zu OA-Patienten und gesunden Probanden signifikant erhöht ($p < 0.0001$). Ebenso fand sich eine signifikante Erhöhung des synovialen IL-34-Spiegels bei den RA-Patienten im Vergleich zu den OA-Patienten ($p = 0.0004$). Die IL-34-Spiegel in Serum und Synovialflüssigkeit waren bei Rheuma faktor (RF)-positiven Patienten ($n = 28$) signifikant höher als bei RF-negativen RA-Patienten ($n = 22$) ($p = 0.03$ bzw. $p = 0.04$). Die IL-34-Spiegel in Serum und Synovialflüssigkeit korrelierten positiv mit dem RF ($r = 0.43$; $p = 0.02$ bzw. $r = 0.39$; $p = 0.03$) und mit dem Ausmaß der radiologischen Schädigung (SENS) ($p = 0.0002$ bzw. $p < 0.0001$). Allerdings zeigte sich keine signifikante Korrelation zwischen den IL-34-Spiegeln und der Krankheitsaktivität.

**Schlussfolgerung** IL-34 scheint eine wichtige Rolle bei der Pathogenese von RA zu spielen und zur Osteodestruktion beizutragen. Daher ist IL-34 ein potenzielles therapeutisches An griffsziel im Behandlungsinstrumentarium der Erkrankung.

### Introduction

Rheumatoid arthritis (RA) is a chronic disease characterized by synovitis, increased inflammatory markers and progressive bone and cartilage erosion [1]. Cytokine imbalance has been considered to play a role in the pathogenesis of RA in several Egyptian studies [2–5].

Another study on Egyptian RA patients reported that IL-33 might be involved in the pathogenesis of the disease and partly contribute to the development of bone erosions [6]. Activated osteoclasts are considered key players in RA pathophysiology, and contribute to focal bone erosion [7, 8]. Osteoclasts (OCs) differentiate from the monocyte/macrophage lineage of hematopoietic myeloid progenitors and the differentiation correlates with the severity of the inflammatory condition [9]. Experiments have shown that macrophage colony stimulating factor (M-CSF) in collaboration with nuclear factor kappa-B ligand (RANKL) has a role in the differentiation of macrophages into osteoclasts, which are involved in bone destruction in RA [10].

Interleukin-34 is a recently discovered pro-inflammatory cytokine, composed of 241 amino acids and having a molecular mass of 39 kDa [11]. IL-34 was shown to have the same role as M-CSF in stimulating colony formation of macrophages in the bone marrow cells. It has been shown that IL-34 can entirely substitute M-CSF in RANKL-induced osteoclastogenesis [12, 13]. IL-34 levels in the synovial fluid of RA joints, were increased by TNF-α stimulated synovial fibroblasts and were thought to be related to the severity of synovitis [14] and have a role in bone destruction [12, 15]. It has been reported that IL-34 could increase IL-6 and chemokine levels in the human whole blood [16]. Furthermore, it was found that IL-34 expression could be induced by TNF-α and IL-1β, which are essential cytokines in RA pathogenesis [17]. Additionally, IL-34 as one of the pro-inflammatory cytokines, could promote inflammation and osteoclastogenesis in arthritic joints, which is the fundamental pathophysiology of RA.

Hence, the aim of the present work was to assess the level of IL-34 in the serum and synovial fluid of RA patients and compare the levels to those of OA patients and healthy patients as control. Additionally, we aimed to understand its correlation with disease activity and with the extent of radiographic joint damage as indicated by the narrowing of joint space and erosions.

### Patients and methods

Serum samples were obtained from 50 consecutive RA patients (40 females, 10 males; F:M = 4:1) who presented to the Rheumatology outpatient clinic of Assuit University Hospitals. All patients met the American College of Rheumatology/European league against rheumatism (ACR/EULAR) criteria for RA [18]. In addition, 28 consecutive OA patients who met the ACR criteria [19] (16 females, 12 males; F:M 1:3:1) and 20 healthy volunteers (12 females and 8 males; F:M 1:5:1) as controls were included. Patients with co-existing infectious diseases, tuberculosis, tumor, hematologic disease, metabolic bone disease, or any other connective tissue diseases were excluded. The study was approved by the ethics committee of our institution according to the Declaration of Helsinki and written informed consent was given by all patients.

For the RA patients, thorough clinical examination was performed and swollen joint count (SJC) and tender joint count (TJC) were recorded. Blood tests included the erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF) titer assessed using a particle-enhanced immunoturbidimetric assay; a level > 15 U/ml was considered positive. The 28-joint count disease activity score (DAS28) was calculated [20]. Disease activity status was defined as remission if DAS28 was < 2.6, low (2.6 ≤ DAS28 ≤ 3.2), moderate (3.2 < DAS28 < 5.1) and high if DAS28 was ≥ 5.1 [21].

The extent of joint damage was radiologically assessed by checking for joint space narrowing (JSN) and erosion, and was evaluated by simplified erosion narrowing score (SENS) [22] which is a simplification of Sharp van der Heijde scoring method. Joint erosions are scored in 32 joints in the hands and wrists and 12 joints in the feet, JSN is assessed in 30 joints hands and wrists, and 12 joints in the feet. A joint is scored as affected (1) if it displays any erosion, and as affected (1) for JSN if it the score is 1 or more in the original method (at least focal [JSN]). The score for each joint can therefore range from 0 to 2. The total SENS score ranges from 0 to 86.

Synovial fluid (SF) samples were obtained consecutively from all RA and OA patients. Serum and SF samples were stored at −80 °C until analysis.

### Cytokine measurement

The IL-34 concentration was measured using an IL-34-specific sandwich ELISA (R&D systems, Minneapolis, MN, USA) in accordance with the protocols of the manufacturer. Recombinant human IL-34 serially diluted in culture media was used as a standard. ELISA plates
were incubated with mouse anti-human IL-34 Ab and then blocked with 3 % BSA in PBS for 1 h at room temperature. SF from patients with RA and OA, or supernatants from FLS cultures were added to the plates. After washing with PBS containing 0.05 % Tween 20, each well was incubated with biotinylated mouse anti-human IL-34 (0.5 μg/ml) for 2 h at room temperature. Plates were then washed with PBS containing 0.05 % Tween 20 and incubated with streptavidin conjugated to horseradish peroxidase (HRP) for 1 h at room temperature. A color reaction was developed with tetramethylbenzidine solution, and then stopped with 0.1 M H2SO4. The absorbance at 450 nm was measured using a Bio-Rad microtiter plate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

Data are provided as range (minimum, maximum), mean ± SD, mean ± SE. Mann-Whitney t test was used to examine the difference between 2 groups with a significance value at p ≤ 0.05. The ANOVA test was used to examine the difference between the groups. Correlations between parameters measured were calculated using Spearman’s correlation coefficient. All statistical analysis was conducted using Graph Pad Prism 6 Software.

Results

The mean age of the patients with RA and OA, and healthy controls was (37.68 ± 12.97, 48.5 ± 11.82, and 30.6 ± 8.38 years, respectively), there was a significant difference (P < 0.002) with age being higher in the "OA" patients, while the gender was comparable among the 3 groups (P = 0.26). The mean RA disease duration was 97.6 months (range 3–300 months). The mean number of deformed joints was 3.37. The DAS-28 mean score was 5.22 ± 0.27; it was high in 52 % and moderate in 44 % patients, while 4 % patients were in remission. The radiological score was 27.38. Demographic, clinical, and laboratory characteristics of the RA patients are presented in ▶ Table 1.

There was a significant difference in the serum IL-34 level between the 3 groups: RA patients (299.7 ± 159.6 pg/ml), OA patients (139 ± 58.1 pg/ml) (P < 0.0002) and healthy controls (73.56 ± 15.8 pg/ml) (P < 0.0001). Similarly, synovial IL-34 level was significantly higher in RA patients (317.2 ± 334.5 pg/ml) compared to that in OA patients (112.7 ± 21.5 pg/ml) (P = 0.0004). The difference in serum and synovial levels in the groups are presented in ▶ Fig. 1. In RA patients, IL-34 concentrations were significantly higher in the serum than in the SF (P < 0.0001). No significant difference in IL-34 level was found between the serum and synovial fluid in OA group. (P = 0.1)

▶ Table 1 Demographic, clinical and laboratory characteristics of the rheumatoid arthritis patients.

<table>
<thead>
<tr>
<th>Characteristic mean ± SD or n (%)</th>
<th>RA patients (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.68 ± 12.97</td>
</tr>
<tr>
<td>F:M</td>
<td>40:10</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>97.60 ± 70.3</td>
</tr>
<tr>
<td>MS (min)</td>
<td>39 ± 37.74</td>
</tr>
<tr>
<td>TJC</td>
<td>8.76 ± 6.03</td>
</tr>
<tr>
<td>SJC</td>
<td>5.92 ± 4.32</td>
</tr>
<tr>
<td>ESR (mm/1st hr)</td>
<td>35.77 ± 21.47</td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>230.1 ± 168.5</td>
</tr>
<tr>
<td>Positive: negative</td>
<td>28 (56 %): 22 (44 %)</td>
</tr>
<tr>
<td>DAS28</td>
<td>5.22 ± 0.27</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
</tr>
<tr>
<td>MTX</td>
<td>34 (68)</td>
</tr>
<tr>
<td>LFN</td>
<td>30 (60)</td>
</tr>
<tr>
<td>SSZ</td>
<td>4 (8)</td>
</tr>
<tr>
<td>HCQ</td>
<td>38 (76)</td>
</tr>
<tr>
<td>Steroids</td>
<td>28 (56)</td>
</tr>
<tr>
<td>SENS</td>
<td>22.80 ± 12.01</td>
</tr>
</tbody>
</table>

RA: rheumatoid arthritis; MS: Morning stiffness, TJC: tender joint count; SJC: swollen joint count; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor; DAS28: Disease Activity Score 28; MTX: methotrexate; LFN: leflunomide; SSZ: sulfasalazine; HCQ: hydroxychloroquine; SENS: simplified erosion narrowing score.

▶ Fig. 1 IL 34 level in serum and synovial fluids of the patient groups. a Synovial IL34 level among RA patients (n = 50) and OA patients (n = 28). b Serum IL34 level among RA patients (n = 50), OA (n = 28) and healthy control group (n = 20).
Serum IL-34 level was significantly \((P = 0.01)\) higher in the RF-positive \((n = 28)\) \((350.3 \pm 158.3 \text{ pg/ml})\) compared to that in RF-negative RA patients \((n = 22)\) \((235.4 \pm 139.7 \text{ pg/ml})\). Similarly, the synovial IL-34 levels in RA patients was significantly \((P = 0.04)\) higher in RF positive cases \((371.9 \pm 338.7 \text{ pg/ml})\) compared to that in RF negative cases \((218 \pm 224.6 \text{ pg/ml})\).

The correlation results of serum and synovial IL-34 levels with the disease parameters are presented in Table 2. There was a significant correlation of both serum and synovial IL-34 levels with the RF \((r = 0.43, P = 0.02)\) and \((r = 0.39, P = 0.03)\), respectively and with the extent of radiological damage (SENS) \((P < 0.0001\) and \(P < 0.0001\), respectively) (Fig. 2). No significant correlation could be detected between the IL-34 levels and parameters of disease activity including TJC, SJC, and DAS28.

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>IL-34 in RA patients ((n = 50))</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Serum</td>
</tr>
<tr>
<td>Age (years)</td>
<td>(-0.11)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>(-0.11)</td>
</tr>
<tr>
<td>TJC</td>
<td>(-0.17)</td>
</tr>
<tr>
<td>SJC</td>
<td>(-0.1)</td>
</tr>
<tr>
<td>ESR</td>
<td>0.27</td>
</tr>
<tr>
<td>RF</td>
<td>0.43</td>
</tr>
<tr>
<td>DAS28</td>
<td>0.05</td>
</tr>
<tr>
<td>SENS</td>
<td>0.53</td>
</tr>
</tbody>
</table>

IL-34: interleukin-34, RA: rheumatoid arthritis; TJC: tender joint count; SJC: swollen joint count; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor; DAS28: Disease Activity Score 28; SENS: simplified erosion narrowing score.; Values are significant at \(p < 0.05\).

### Discussion

Rheumatoid arthritis is characterized by subchondral bone loss, and the mechanism of bone loss is similar or identical to that in osteoporosis [23]. Pro-inflammatory cytokines promoting inflammation and osteoclastogenesis in the arthritic joint are fundamental to RA pathophysiology [24]. It has been revealed that IL-34 expression increases in the synovium, serum, and SF of RA patients and is associated with RF and anticyclic citrullinated peptide (CCP) antibody titers [14, 25]. A significant role of IL-34 in RA synovitis has been suggested [26] and functionally isolated RA-derived fibroblast-like synoviocytes and osteoblasts were found to produce IL-34 in response to TNF-α [16].

In this study, serum IL-34 levels were significantly higher in RA patients than in those with OA and controls. The same results were reported by Zhang et al. [27], Hwang et al. [15], Moon et al. [25], and Tian et al. [17]. The concentration of IL-34 in SF was also higher in RA patients than in patients with OA as reported by Moon et al. [25] and Chang et al. [28]. These results support the hypothesis that IL-34 might play a pathophysiological role in RA.

In the present study, serum IL-34 levels in OA patients was significantly higher than that in healthy controls. There is compelling evidence that various inflammatory cytokines participating in the pathogenesis of OA, and IL-1β, TNF-α and IL-6 are the most important pro-inflammatory mediators in the development of OA disease [29].

Also, Elevation of IL-34 levels can occur in other inflammatory conditions. For instance, IL-34 expression was increased in the salivary gland of Sjogren’s syndrome patients, in patients with coronary artery disease [30, 31] and in Psoriatic arthritis patients compared to non-arthritic ones and its remarkable correlation with Psoriatic arthritis disease activity [32].

Serum IL-34 concentration in RA patients significantly correlated with RF titers. The same was reported by Moon et al. [25] and Tian et al. [17], suggesting that IL-34 might contribute to autoantibody production in RA. Until now, there is no evidence suggesting any direct link between T or B-lymphocyte and IL-34. Therefore, to understand the mechanism explaining the relationship between IL-34 and RF, further studies are needed.
and autoantibody titers, further research is necessary. In contrast; RA disease activity did not show any significant correlation with IL-34 values in RA patients as reported in the studies of Chemel et al. [14] and Chang et al. [28]. Furthermore, Moon et al. found no correlation between IL-34 with the clinical or laboratory parameters of disease activity. Considered together, these data indicate that serum IL-34 level does not reflect the inflammatory status in RA and caution should be exercised when including it as a measure of disease activity. On the other hand, Tian et al. [17] found a significant correlation between IL-34 levels and disease activity indices, including TJC and DAS28.

Based on previous studies, IL-34 has been detected in the synovial biopsy and has been found to be related to bone erosion in RA pathogenesis [12, 15]. In order to confirm the role of IL-34 in bone destruction without the use of invasive examinations, the extent of radiographic damage using x-ray radiographic scores was measured and the scores showed a significant correlation with IL-34 values, indicating that it might contribute to the development of bone erosions in RA. This was in accordance with a recent study by Chang et al. that found a significant correlation of baseline IL-34 levels with the radiographic progression as assessed by the Sharp-van der Heijde Score [28]. However, this was in contrast to the findings of Moon et al., who found no significant correlation between serum IL-34 levels and the extent of radiographic damage although its concentration in synovial fluid correlated with the RANKL levels [25].

**Conclusion**

In conclusion, this study has found increased serum and SF levels of IL-34 in patients with RA. We have shown that IL-34 was significantly associated with autoantibody production and bone destruction. This supports the hypothesis that IL-34 might contribute to bone destruction in RA, since IL-34 induces osteoclastogenesis and represents a potential future therapeutic target for RA. Further studies with a larger sample size that evaluate other cytokines and autoantibodies as well are needed to clarify the exact role of IL-34 in the inflammatory process associated with RA.

**Conflict of Interest**

The authors declare no conflict of interest.

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