Assessment of synovitis in early rheumatoid arthritis by CXCL13 serum levels and power Doppler ultrasonography: Correlation with disease activity

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Abstract Aim of the work: Assessment of synovitis in rheumatoid arthritis (RA) is a major issue for proper treatment; it has been proven that high resolution ultrasound (US) examination could be of valuable help. The B-cell chemokine, CXCL13, is a proposed serum biomarker of synovitis in RA. We aimed to find out the presence of synovitis in patients with recent-onset RA and its correlation with disease activity.

Patients and methods: We evaluated 30 patients with early RA for the presence and degree of synovitis by performing high resolution US and obtaining serum CXCL13 levels. In addition, we correlated these results with disease activity score 28 (DAS 28). Results of high resolution US and serum CXCL13 were also obtained for 20 healthy age- and sex-matched volunteers and served as controls.

Results: Serum CXCL13 level was significantly increased in early RA patients vs. controls (p < 0.001). High resolution US revealed that RA patients had a significant increased synovial thickness and high power Doppler US score. In RA patients, DAS 28 had a significant correlation with serum CXCL13 (r = 0.42, p = 0.02), synovial thickness (r = 0.39, p = 0.03) and power Doppler US score (r = 0.43, p = 0.02). Serum CXCL13 level correlated with synovial thickness (r = 0.63, p = 0.001) and power Doppler US score (r = 0.69, p = 0.001).

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1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease targeting multiple joints. The synovium is the primary site of the inflammatory process, which if untreated leads to irreversibly damage to the adjacent cartilage and bone [1]. This process is characterized by an increased presence of monocytes, macrophages, and lymphocytes in the synovial fluid and tissue, leading to the release of cytokines and chemokines. These proinflammatory mediators subsequently activate different proteases, culminating in the destruction of bone and cartilage, which leads to increased disability in RA patients [2].

Serum levels of the B-cell attracting chemokine, CXCL13 have been proposed as a marker of synovitis in rheumatoid arthritis [3,4]. CXCL13 and its receptor CXCR5 serve a homeostatic role in the immune system. CXCL13 is constitutively expressed in the B-cell areas of secondary lymphoid tissues such as spleen and lymph nodes [5]. CXCL13 directs circulating B cells and follicular T-helper cells to home to the B-cell area during lymphoid organ development and routine immune surveillance [6].

In addition, CXCL13 and CXCR5 also participate in the pathogenesis of certain chronic inflammatory diseases. Under these conditions, CXCL13 expression is induced at nonlymphoid inflammatory sites and participates in ectopic germinal center formation. These ectopic germinal centers provide an optimal microenvironment for B-cell activation, differentiation, maturation, and autoantibody generation, resulting in accelerated disease progression [7].

Rheumatoid arthritis (RA) is characterized by synovial inflammation and bone and cartilage destruction. While synovial inflammation is typically assessed by physical examination, measurement of bone and cartilage damage is a domain of radiography. In recent years, high-resolution ultrasound (US) has been increasingly used to assess synovial inflammation [8]. With US, one can quantitatively assess the morphologic (synovial thickness) and functional (blood flow) changes of joints during inflammatory arthritis [9].

Clinical trials concerning RA are based upon various composite indices for assessing disease activity like disease activity score 28 (DAS28), health assessment questionnaire (HAQ), clinical disease activity index (CDAI) and the simplified disease activity index (SDAI). They have proven sensitivity to change, validity and reliability; unfortunately they are mainly based on subjective issues like patient’s appreciation of pain, while, US was proven to be better than clinical examination in detecting synovitis [10].

Therefore, we aimed to assess the presence and severity of synovitis in patients with early rheumatoid arthritis by high resolution power Doppler ultrasonography and by measuring serum CXCL13 and to correlate these findings with the clinical disease activity.

2. Patients and methods

Thirty patients with early RA (disease duration less than 1 year) were selected from those presenting to the Internal Medicine and Physical Medicine, Rheumatology, and Rehabilitation outpatient clinics of the Ain Shams University Hospitals. RA patients were all fulfilling the new EULAR/ACR criteria for RA. A score of $\geq 6/10$ is needed for classification of a patient as having definite RA [11]. All patients had clinical arthritis during examination. They were on methotrexate, folinic acids and hydroxychloroquine, in addition to corticosteroids during flaring up of the disease.

The control group consisted of 20 healthy, age- and gender-matched volunteers. The study protocol was in accordance with Helsinki declaration of human rights, and was approved by the local Ethics Committee. The written informed consent from each patient and control was obtained.

All patients were subjected to the following:

1. Full history taking and thorough physical examination including detailed musculoskeletal examination.
2. Assessment of disease activity by using the modified DAS-28 score. A patient with DAS-28 score $\leq 2.6$ is considered in remission, from 2.6 to $\leq 3.2$ = low disease activity, from 3.2 to $< 5.1$ = moderate disease activity and $\geq 5.1$ = high disease activity [12].
3. Functional assessment was done by using the health assessment questionnaire (HAQ) score (validated Arabic version) [13].
4. Radiological examination:
   - Plain X-ray of hands was done on all RA patients and X-ray score was calculated according to Larsen et al. [14].
   - High resolution US using a gray-scale US mode (13–1-8 MHz), Philips HD 11, by an expert radiologist was done on all patients and controls. The patient was seated with hands lying in prone position on the examination table. Both longitudinal and transverse scans were performed by slightly moving the transducer from radial to ulnar and from proximal to distal sides on the dorsal aspect to enable maximum coverage of the anatomical surface areas. The sites that were assessed by US included the dorsal regions of the 2nd MCP joint, 3rd MCP joint, and the dorsal regions of the wrist [15]. The intraarticular, tenosynovial and intrabursal power Doppler (PD) signals were graded on a semi-quantitative scale from 0 to 3 (grade 0 = absence, no synovial flow; grade 1 = mild, < 3 isolated signals; grade 2 = moderate, > 3 isolated signals or confluent signals in less than half of the synovial area; grade 3 = marked, signals in more than half of the synovial area). These scores corresponded to the maximum score for PD signals obtained from any of the synovial
sites evaluated at each joint. The sum of the PD signal scores obtained from each joint was used as the PDUS score, as reported by Naredo et al. [10]. For better assessment of synovitis by US, the number of scanned joints should range from 6 to 12 joints [16]. For better clinical availability, Kawashiri et al. [15] and Taylor et al. [17] reduced the number of joints examined by US to only six sites of the wrist and finger joints. Since the second and the third metacarpophalangeal (MCP) joints showed the most radiological findings as regarded by Naredo et al. [10], we have chosen six synovial sites from six joints including the bilateral wrists (dorsal recess) and second and third MCP joints (dorsal recess). The six joint (6j)-PDUS score was the sum of the six synovial sites.

5. Laboratory investigations
- Complete blood count (CBC), Erythrocyte sedimentation rate (ESR) in first hour in mm/hr.
- Serum anti CCP antibodies level was assessed by ELISA using QUANAT Lite™ CCP3 IgG semiquantitative ELISA kit, INOVA Diagnostics, Inc., San Diego, CA, USA. Serum samples of anti CCP antibody levels less than 20 U/ml were considered negative, and designated as the “standard” cut off.
- Rheumatoid factor (IgM) was measured by Biotec, that was the factor latex agglutination slide for qualitative determination of RF in serum.
- Serum CXCL13 level was quantitatively determined using Human CXCL13 enzyme linked immunosorbent assay (ELISA) (RayBio®, RayBiotech, Inc., USA) [18].

Statistical methods: IBM SPSS statistical software package (V. 19.0, IBM Corp., USA, 2010) was used for data analysis. Data were expressed as range (minimum–maximum), mean ± SD and median for quantitative measures and both number and percentage for categorical data. Comparison between two independent groups of numerical parametric data was done using Student’s t-test. Ranked Spearman correlation test was done to study the possible association between each of the two variables among each group for nonparametric data. Probability of error at <0.05 was considered significant and highly significant at <0.001.

3. Results

This study included 30 patients with early rheumatoid arthritis (27 females and 3 males). Twenty healthy subjects (18 females and 2 males) served as the control group. The patients’ age ranged from 25 to 59 years, with a mean of 37.8 ± 10.1 years. The dis- this was matched with the age of the control group that ranged from 27 females and 3 males). Twenty healthy subjects (18 females and 2 males) served as the control group. The patients’ age ranged from 25 to 59 years, with a mean of 37.8 ± 10.1 years. The dis-

Table 1 Clinical data of RA patients.

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>RA patients (n = 30)</th>
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<tbody>
<tr>
<td>Min.–Max.</td>
<td>Median</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>DAS 28</td>
<td>3.35–7.32</td>
</tr>
<tr>
<td>HAQ</td>
<td>1–3</td>
</tr>
<tr>
<td>Score of diagnosis</td>
<td>6–8</td>
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</table>

2.7 ± 0.5 (Table 1). Plain X-ray score (Larsen score) ranged from 0 to 2 with a mean of 1.5 ± 0.7.

High resolution US revealed highly significant increased synovial thickness (p < 0.001) in the examined 6 joints that ranged from 0.7 to 4.1 mm with a mean of 1.8 ± 0.95 mm in comparison to the control group that ranged from 0.3 to 0.5 mm with a mean of 0.4 ± 0.08 mm.

Power Doppler US score measured in each joint was grade 1 or 2 in all patients (no marked grade; grade 3 in any examined joint). So, power Doppler US score measured in all 6 joints of RA patients ranged from 6 to 12 with a mean of 8.37 ± 2.15. On the other hand, power Doppler US score in the control group was 0 in all 6 joints.

ESR levels ranged from 20 to 120 mm/h in RA patients with a mean of 45.47 ± 26.25 mm/h. Rheumatoid factor was positive in 26 RA patients (86.7%). While, serum anti CCP antibodies was positive in 25 RA patients (83.3%).

Serum CXCL13 levels ranged from 120 to 350 pg/ml in RA patients. When compared with a range of 8–30 pg/ml in the control group, there was a highly significant elevation in serum CXCL13 levels in RA patients versus controls (p < 0.001), (Table 2 and Fig. 1).

In RA patients, DAS 28 score showed significant correlation with serum CXCL13 levels (r = 0.42, p = 0.02) and also with synovial thickness (r = 0.39, p = 0.03) and power Doppler US score (r = 0.43, p = 0.02).

On the other hand, the serum levels of CXCL13 in RA patients correlated with the synovial thickness (r = 0.63, p = 0.001) (Fig. 2) and with PDUS score (r = 0.69, p = 0.001) (Fig. 3), while there was an insignificant correlation between serum CXCL13 levels and either ESR, HAQ or Larsen score (p > 0.05) Figs. 4–6 show ultrasonographic findings in RA patients and controls.

4. Discussion

Serum levels of the B-cell attracting chemokine, CXCL13 have been proposed as a marker of synovitis, and predicted synovitis outcomes in patients with recent-onset RA [4,18]. In this study, we aimed to find out the presence and severity of synovitis in early rheumatoid arthritis patients by high resolution power Doppler US and by measuring serum CXCL13 and correlate these findings with the disease activity.

There may be many sources of serum CXCL13, including lymph nodes [19]. Manzo et al. [20] reported that serum CXCL13 levels can be changed in association with active RA. So, synovitis appears to significantly contribute to the systemic up-regulation of CXCL13 [3]. Furthermore, during inflammation, the released interleukins induce the production of acute phase proteins from hepatocytes [21]. The assessment of CXCL13 levels in the peripheral circulation may be used as

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a comprehensive, non-invasive tool to gain further information on the clinical significance of synovitis in RA [22].

Our results showed that serum levels of CXCL13 were significantly higher in RA compared to healthy controls. This was in agreement with Bugatti et al. [18] and Manzo et al. [23], whose studies were also on early RA (disease duration less than 12 months).

In the present study, we used three scoring systems; clinical score (DAS 28), functional score (HAQ) and radiological scoring system to assess the disease activity, the related disability and the extent of synovitis respectively in early RA patients.

### Table 2  Comparison between RA patients and controls as regards serum CXCL13 levels.

<table>
<thead>
<tr>
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<th>RA patients (n = 30)</th>
<th>Controls (n = 20)</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>Min.–Max. Median Mean ± SD</td>
<td>Min.–Max. Median Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>CXCL13 (pg/ml)</td>
<td>120–350 145 165.4 ± 49.4</td>
<td>8–30 15 15.7 + 5.9</td>
<td>0.001</td>
</tr>
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</table>

$r = 0.63, p = 0.001$

X axis represents CXCL 13, Y axis represents synovial thickness.

Figure 1  Comparison between RA patients and controls as regards serum CXCL13 levels.

$r = 0.69, p = 0.001$

X axis represents CXCL 13, Y axis represents power Doppler score.

Figure 2  Correlation between serum CXCL13 levels and synovial thickening as measured by high resolution US in RA patients.

Figure 3  Correlation between serum CXCL13 levels and PDUS score in RA patients.
DAS 28 was significantly correlated with serum CXCL13 levels and also with measures of synovitis; synovial thickness and power Doppler US score. Other studies showed significant association between serum CXCL13 levels and either objective or semi-objective measures of disease activity, such as the swollen joint count, the evaluator global assessment of disease activity, the erythrocyte sedimentation rate and C-reactive protein levels [23,24]. Also Kawashiri et al. [15] revealed that DAS-28 was of better correlation with the PDUS score than SDAI and CDAI, and these data reinforce the validity of PDUS for the measurement of the disease activity of RA.

As regards the functional score, there was insignificant correlation between HAQ and either serum CXCL13 levels or measures of synovitis. This may be explained by two reasons; first: the short disease duration of our patients (less than one year) and the second is that not one of our patients had severe synovitis (grade 3 by power Doppler US).

It is widely accepted now that US is more accurate than clinical examination in detecting synovitis [10], and PD technologies further improve the capability of identifying actively inflamed joints [25]. We agree with Backhaus et al. [26] that power Doppler US is a noninvasive, relatively inexpensive bed-
side imaging modality that facilitates the scanning of all peripheral joints as many times as required.

We found that serum levels of CXCL13 correlated with the severity of synovitis as assessed by US (synovial thickness and PDUS score). Thus, measurement of CXCL13 levels may help to predict active joint inflammation that was detected by power Doppler US and help in the assessment of the degree of synovitis compared to other routine markers (acute phase proteins as ESR and C reactive proteins) that are only indirectly linked to joint inflammation. Also, Bugatti et al. in 2012 [18] found that serum CXCL13 was significantly correlated with the baseline PD scores and serum CXCL13 levels emerged as the only baseline predictor of US outcomes in their cohort in comparison to CRP or ESR serum levels.

There was no significant correlation between serum CXCL13 levels and the Larsen score in our cohort. On the other hand, Meeuwisse’s et al. [27], showed that higher CXCL13 serum levels were reported to be associated with increased rates of joint destruction in their long term study.

In conclusion, synovial hypertrophy in early RA patients can be detected by high resolution power Doppler US. The use of power Doppler US in assessing early synovial hypertrophy is a non invasive, easily used and reproducible tool for the evaluation of the severity of synovitis in these patients. Serum CXCL13 can be used as an early new biological marker that gives an idea about the severity of synovitis and activity of the disease. Serum CXCL13 levels correlated better with synovial thickening and power Doppler score than DAS 28. So, screening of early RA patients for synovitis is recommended by either measuring serum CXCL13 or by high resolution US for better assessment and management of the disease.

Conflict of interest

No conflict of interest.

Acknowledgement

The authors would like to thank Dr. Amal Abbas, Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for technical support.

References


Figure 6  Transverse scan of the dorsal aspect of the right wrist joint of RA patient showing moderate arthro-synovial proliferation (black arrow) in (a) with moderate tenosynovitis of the extensor carpi ulnaris tendon (white arrow) shown in (b), with mild vascularity, grade 1 as shown in (c).
Assessment of synovitis in early rheumatoid arthritis by CXCL13 serum levels


