The significance of interferon-inducible protein-16 and anti-interferon-inducible protein-16 antibodies in rheumatoid arthritis: Relation to pulmonary disease and serological markers

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Abstract

Aim
The aim of the study was to detect the presence of interferon-inducible protein-16 (IFI16) as well as anti-IFI16 antibodies in the serum and synovial fluid of patients with rheumatoid arthritis (RA) and to evaluate the relation to pulmonary interstitial lung disease (ILD) patterns, disease activity, joints erosions, and serological markers.

Patients and methods
This study involved two groups: 82 adult patients with established RA participated as a study group, whereas 30 patients with knee osteoarthritis also joined as a control group. IFI16 and anti-IFI16 immunoglobulin G were assessed by enzyme-linked immunosorbent assay in serum of all patients and controls, whereas both markers were assessed in synovial fluid withdrawn from 36 patients with RA patients and 14 patients of controls. High-resolution computed tomography was used for assessment of pulmonary involvement.

Results
Serum and synovial levels of IFI16 protein and anti-IFI16 antibodies were significantly elevated in patients with RA than controls ($P < 0.001$ and $P = 0.02$, respectively). Patients with RA with pulmonary involvement showed higher serum level of IFI16 protein as well as anti-IFI16 antibody titer than other patients ($P < 0.001$ and $P = 0.005$, respectively). No significant differences were reported between different patterns of RA-ILD regarding serum level of IFI16 protein or anti-IFI16 antibody titer. The latter markers were positively correlated with anti-cyclic citrullinated peptide antibody titer ($P < 0.001$ and $P = 0.037$, respectively).

Conclusion
Serum IFI16 and anti-IFI16 antibodies could be used as valuable indicators of RA-related pulmonary ILD but larger studies are needed to confirm it.

Keywords: Anti-interferon-inducible protein-16 antibodies, interferon-inducible protein-16, rheumatoid arthritis-related interstitial lung disease

INTRODUCTION

Type I interferons (IFNs) are considered as the first-line defense mechanism in innate immune response that can also magnify adaptive immune response [1]. These IFNs perform plenty of functions including development, maturation, and survival of dendritic cell [2], B cell, and T cell [3,4], in addition to its role in antiviral response [5,6].
Overexpression of type I IFN-stimulated genes is a cornerstone of systemic lupus erythematosus [7] and other IFN-driven diseases, such as primary Sjogren’s syndrome [8], systemic sclerosis, and rheumatoid arthritis (RA) [9].

Regarding RA, the expression of type I IFN genes is increased in early and presymptomatic arthritis and enhances breakage of immune tolerance to self-antigen and development of autoimmunity following viral infection [10,11]. However, in a well-established disease, type I IFNs play a role in disease perpetuation through Th2 activation, B cells proliferation with production of autoantibodies, and increased resistance to apoptosis [12–17]. Baseline IFN-stimulated genes may influence the response to biological therapy [18].

Interferon-inducible protein-16 (IFI16) is a product of IFI16 gene, a member of HIN200/IFI200 family of genes [19]. It comprises two domains: HIN domain and N-terminal PYRIN domain (or DAPIN domain) [20].

This protein is located in the nucleoplasm, and through its domains, it can perform several effector functions in transcription regulation, apoptosis, and antiviral response [21–23]. IFI16 has been found to sense nuclear and cytoplasmic foreign DNA during viral infection. By binding of HIN200 domains to dsDNA, IFI16 upregulates stimulator of interferon genes (STING) followed by phosphorylation of interferon regulatory factor-3 through TANK-binding kinase-1 and finally transcription of type I IFNs [24].

There is growing evidence suggesting the presence of such intracellular protein, (IFI16), in the circulation of auto immune diseases, including RA, where it exerts multiple pathological processes and is a source of autoantigens accompanied by autoantibodies production [25–28].

Additionally, IFI16 was found to be related to certain extra-articular manifestations, especially pulmonary involvement, and it may be considered as a viable marker of RA-associated pulmonary lung interstitial disease (ILD) [29].

So, the aim of the study was to detect the presence of IFI16 as well as anti-IFI antibodies in the serum and synovial fluid of patients with RA and to evaluate whether it is related to certain patterns of pulmonary ILD in RA and to find out the relation to disease activity, joint erosions, and serological markers.

**Patients and methods**

A total of 82 adult patients with established RA [30] (with disease duration >1 year) participated in this cross-sectional observational study. Moreover, 30 age-matched and sex-matched patients with knee osteoarthritis also joined as a control group. The patients’ recruitment started in May 2018, and the study was completed in April 2019.

Before involvement in the study, the patients signed an informed consent according to the Helsinki Declaration criteria [31] and after approval from the local ethics committee (GOTHI).

The patients who showed the following conditions were excluded from the study: pregnancy and lactation, previous intra-articular injection (corticosteroids or hyaluronic acid) within the past 6 months before the beginning of the study, septic arthritis, treatment with biological agents, other autoimmune diseases or chronic pulmonary disorders, possibilities of drug-induced ILD [32–34], acute and chronic infections including tuberculosis, renal disease, hepatic diseases, or other chronic comorbidities including malignancy. The latter criteria were applied on the control group.

Thorough clinical, laboratory, and radiologic assessments were performed for all patients with RA. Disease activity was assessed using disease activity score with 28-joint count [35] and joint erosion grades were assessed using modified Larsen’s score [36]. Blood samples were withdrawn from patients and controls. A part of those samples was used for assessment of erythrocyte sedimentation rate, whereas the other part was left to clot for 10–20 at room temperature, and then centrifugation was done at 2000–3000 RPM for 20 min.

Synovial fluid samples were aspirated from the knee joints of 36 patients with RA and 14 patients of controls under complete aseptic conditions.

The serum and synovial samples were stored at −80°C until the time of assessment of other laboratory parameters.

**Assessment of human interferon-inducible protein-16**

Serum and synovial levels of human IFI16 protein was assessed by sandwich enzyme-linked immunosorbent assay technique (Bioassay Technology Laboratory, Shanghai China, Cat. No E1957Hu, standard curve range, 10–2000 ng/l). The principles of assay included particular steps according to manufacturer instructions. First, human IFI16 protein present in the tested samples was added to microwells containing monoclonal antibodies against human IFI16. After incubation, biotin-conjugated anti-human IFI16 antibodies were added to react with IFI16 present in the wells. This combination was incubated for another time followed by washing to eliminate any unbound reagents. Then streptavidin–HRP was added to interact with biotin-conjugated anti-human IFI16 antibodies followed by incubation. In the second washing process, any unbound reagents were also eliminated. Finally, a substrate is added followed by color development according to the concentration of human IFI16 protein. This reaction is stopped by adding an acidic solution. Absorbance was measured at 450 nm.

**Assessment of anti-human interferon-inducible protein-16 antibodies**

By using enzyme-linked immunosorbent assay kits (DiaMetra, Perugia Italy), a semiquantitative measurement of anti-human IFI16 immunoglobulin G antibodies was performed in the serum and synovial fluid according to manufacturer’s instructions. The principles of the study are based on addition of diluted samples containing anti-human IFI16 to microwells that are precoated with IFI16 antigen. After incubation for
30 min, through washing step, nonreactive serum elements were removed. Then an anti-human-immunoglobulin G horseradish peroxidase conjugate solution was added to bind the immobilized antigens in the first step. This combination was incubated for 30 min followed by another washing step. A solution containing TMB, a substrate with chromogenic characteristics, was added to the wells, with color development after 15 min incubation. An acidic solution was used to stop color development. According to optical density of the color, the concentration of immunoglobulin G anti-IFI16 antibodies present in the tested sample was measured. The range for antibody concentration according to manufacturer’s reference is as follows: when anti-IFI16 antibodies concentration was less than 80 U/ml, the test is considered negative, whereas above 80 U/ml, the test is positive

**High-resolution computed tomography of the chest**

Pulmonary involvement was assessed using high-resolution computed tomography [Healthcare GE Revolution Evo 64 ASIR (adaptive statistical iterative reconstruction), Madison, Wisconsin, USA]. The patients were examined in supine position. Series I involves PA and lateral scout views, from the base of the neck through the lung bases. Series II scout views are from just above the lung apices and extend through the 12th rib, so as to image the entire bony thorax. The scan was taken with inspiration. Slice thickness was 1 mm, and scan time was about 5 s. Coronal and sagittal reformats were added on chest protocol (axial cuts) as well as mediastinal window. The abnormalities were evaluated by an expert radiologist (Dr H.A.E.T) without any knowledge about clinical data of the patients.

**Statistical analysis**

The results were analyzed using SPSS, version 16, software (SPSS Inc., Chicago, Illinois, USA). Shapiro–Wilks test was used to assess normality of data \( P > 0.05 \). Mann–Whitney \( U \) test \( (Z_{MWU}) \) was used to analyze difference among two independent groups regarding not normally distributed variables, whereas Kruskal–Wallis test was used for three independent groups. For assessment of correlation between the data, Spearman’s correlation coefficient (rho) was used. The cutoff value of IFI16 protein and antibodies in prediction of pulmonary involvement among patients with RA was detected using receiver operating characteristic curve. The relation between different data is considered significant if \( P \) value less than 0.05.

**Results**

This work included 82 patients with RA, comprising 75 females and seven males. Their ages ranged from 25 to 65 years (mean ± SD, 46.32 ± 10.02), with disease duration ranging from 2 to 30 years (mean ± SD, 11.55 ± 6.88). All of them were nonsmokers. All patients with RA were on usual DMARDs therapy. Most of those patients showed an active disease (according to disease activity score with 28-joint count, it ranged from 1.75 to 6.51, with mean ± SD, 3.85 ± 1.167) and seropositivity for both RF (it range, 17.65–491.79, with mean ± SD, 104.54 ± 108.38 U/ml) and anti-cyclic citrullinated peptide (anti-CCP antibodies) titers (it range, 21.02–911.73, with mean ± SD, 291.34 ± 230.93 U/ml).

Within the RA patient group, 52 patients showed extra-articular manifestations besides the joint disease. Pulmonary involvement was observed in 40 patients (37 females and three males, 48.78%), whereas other extra-articular manifestations were observed in 12 patients (10 patients with subcutaneous nodules, one patient with peripheral neuropathy, and one patient with vasculitic ulcer). Among 40 patients with pulmonary involvement, 32 patients presented clinically with chronic dyspnea, chest pain, or nonproductive cough, whereas eight patients were nonsymptomatic. High-resolution computed tomography showed different radiological patterns; 26 patients exhibited radiological findings suggestive of usual interstitial pneumonia (UIP) (fine interstitial reticulations, traction bronchiectasis, and honey-combing appearance with peripheral and basal predominance), whereas 11 patients showed a pattern suggestive of nonspecific interstitial pneumonia (NSIP) (diffuse, peripheral, basal, ground-glass opacities, and irregular linear opacities) (Fig. 1), in addition to a mixed pattern that was detected in three patients, and one of them also showed pulmonary rheumatoid nodules (Fig. 2).

IFI16 protein and anti-IFI16 antibodies were detected in the serum and synovial fluid samples of patients with RA, and their levels were significantly elevated than found in control group \( (P < 0.001 \) and \( P = 0.02, \) respectively) (Tables 1 and 2, Fig. 3).

In patients with RA, the level of IFI16 protein was positively correlated with anti-IFI16 antibodies titer in the serum and synovial fluid samples \( (r = 0.350, P = 0.001, \) and \( r = 0.377, P = 0.024, \) respectively). However, there is poor correlation between serum and synovial fluid levels of each marker \( (r = 0.034, P = 0.844, \) for IFI16 and \( r = 0.201, P = 0.239, \) for anti-IFI16 antibodies).

**Figure 1:** Plain HRCT of the chest of a 55-year-old female patient with RA (pulmonary window, axial cut) revealed diffuse bilateral ground-glass opacities with interseptal thickening. HRCT, high-resolution computed tomography; RA, rheumatoid arthritis.
Patients with RA with pulmonary involvement showed higher serum level of IFI16 protein as well as anti-IFI16 antibody titer than patients with other extra-articular manifestations or patients with pure joint disease ($P < 0.001$ and $P = 0.005$, respectively) (Table 3). However, there is no significant difference between patients presented with UIP pattern and patients presented with other patterns of pulmonary disease regarding serum level of IFI16 protein or anti-IFI16 antibody titer ($P = 0.18$). Serum IFI16 level and anti-IFI16 antibodies titer were positively correlated with anti-CCP antibodies titer ($P < 0.001$ and $P = 0.037$, respectively), whereas both serum and synovial levels of the protein and its antibody did not correlate with demographic, clinical, radiological, or other laboratory parameters of patients with RA (Tables 4, 5). The cutoff value of serum IFI16 for prediction of pulmonary involvement in patients with RA was more than or equal to 878.1 ng/ml (sensitivity 82.5%, specificity 73.8%, positive predicted value 75%, negative predicted value 81.65%, accuracy 78%, area under the curve 0.868, 95% confidence interval 0.79–0.95, and $P < 0.001$), whereas the cutoff value

### Table 1: Serum and synovial interferon-inducible protein-16 and anti-interferon-inducible protein-16 antibodies among the studied groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients ($n=82$)</th>
<th>Controls ($n=30$)</th>
<th>ZMWU test</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IFI16 (ng/ml)</td>
<td>856.6±157.0</td>
<td>83.9±5.9</td>
<td>8.08</td>
<td>&lt;0.001 (HS)</td>
</tr>
<tr>
<td>Serum anti-IFI16 Ab titer (u/ml)</td>
<td>171.5±131.3</td>
<td>55.8±27.3</td>
<td>3.85</td>
<td>&lt;0.001 (HS)</td>
</tr>
<tr>
<td>Synovial IFI16 (ng/ml)</td>
<td>1018.7±74.6</td>
<td>79.5±7.08</td>
<td>5.44</td>
<td>&lt;0.001 (HS)</td>
</tr>
<tr>
<td>Synovial IFI16 Ab titer (u/ml)</td>
<td>225.0±162.2</td>
<td>61.0±20.0</td>
<td>2.34</td>
<td>0.02 (S)</td>
</tr>
</tbody>
</table>

Ab, antibody; HS, highly significant; IFI16, interferon-inducible protein-16; MWU, Mann-Whitney U test; S, significant.

### Table 2: Interquartile range (25th-75th percentiles) of interferon-inducible protein-16 protein and anti-interferon-inducible protein-16 antibodies in the studied groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients ($n=82$)</th>
<th>Controls ($n=30$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IFI16 (ng/ml)</td>
<td>881.1</td>
<td>83.1</td>
<td>&lt;0.001 (HS)</td>
</tr>
<tr>
<td>Serum anti-IFI16 Ab titer (u/ml)</td>
<td>87.0</td>
<td>67.0</td>
<td>&lt;0.001 (HS)</td>
</tr>
<tr>
<td>Synovial IFI16 (ng/ml)</td>
<td>1020.3</td>
<td>80.8</td>
<td>&lt;0.001 (HS)</td>
</tr>
<tr>
<td>Synovial IFI16 Ab titer (u/ml)</td>
<td>234.0</td>
<td>66.5</td>
<td>0.02 (S)</td>
</tr>
</tbody>
</table>

Ab, antibody; HS, highly significant; IFI16, interferon-inducible protein-16; IQR, interquartile range; S, significant.

### Table 3: Serum interferon-inducible protein-16 and anti-interferon-inducible protein-16 antibodies according clinical manifestations and radiological findings

<table>
<thead>
<tr>
<th></th>
<th>$n$</th>
<th>Mean±SD</th>
<th>Range</th>
<th>Minimum</th>
<th>Maximum</th>
<th>ZMWU test</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IFI16 (ng/ml)</td>
<td>Pulmonary</td>
<td>40</td>
<td>948.3±57.0</td>
<td>420-1100</td>
<td>791.3</td>
<td>1100.1</td>
<td>33.6</td>
</tr>
<tr>
<td></td>
<td>Other systems</td>
<td>12</td>
<td>819.9±64.2</td>
<td>642</td>
<td>720.6</td>
<td>900.4</td>
<td>3.85</td>
</tr>
<tr>
<td></td>
<td>Non</td>
<td>30</td>
<td>748.9±181.8</td>
<td>181.8</td>
<td>420.2</td>
<td>1070.7</td>
<td>-</td>
</tr>
<tr>
<td>Serum anti-IFI16 Ab titer (u/ml)</td>
<td>Pulmonary</td>
<td>40</td>
<td>229.8±52.7</td>
<td>135.1</td>
<td>28.0</td>
<td>458.0</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>Other systems</td>
<td>12</td>
<td>116.3±103.6</td>
<td>103.6</td>
<td>29.0</td>
<td>311.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Non</td>
<td>30</td>
<td>115.9±101.8</td>
<td>101.8</td>
<td>27.0</td>
<td>354.0</td>
<td>-</td>
</tr>
<tr>
<td>Serum IFI16 (ng/ml)</td>
<td>UIP</td>
<td>26</td>
<td>933.6±79.7</td>
<td>79.7</td>
<td>791.34</td>
<td>1100.10</td>
<td>ZMWU</td>
</tr>
<tr>
<td></td>
<td>Other patterns</td>
<td>14</td>
<td>975.6±95.6</td>
<td>95.6</td>
<td>875.20</td>
<td>1100.25</td>
<td>-</td>
</tr>
<tr>
<td>Serum anti-IFI16 Ab titer (u/ml)</td>
<td>UIP</td>
<td>26</td>
<td>233.3±140.9</td>
<td>140.9</td>
<td>32.00</td>
<td>458.00</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Other patterns</td>
<td>14</td>
<td>223.1±128.3</td>
<td>128.3</td>
<td>28.00</td>
<td>457.00</td>
<td>-</td>
</tr>
</tbody>
</table>

Ab, antibody; HS, highly significant; IFI16, interferon-inducible protein-16; KWT, Kruskal-Wallis test; MWU, Mann-Whitney U test; S, significant; UIP, usual interstitial pneumonia. *Other systemic manifestations include rheumatoid nodules (10 patients), peripheral neuropathy (one patient), and vasculitic ulcer (one patient). ^Pure articular disease. "Nonspecific interstitial pneumonia and mixed patterns. *Significant in comparison with 'other system.' +Significant in comparison with 'non.'
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of serum level of anti-IFI16 antibodies for prediction of pulmonary involvement in patients with RA was more than or equal to 88.5 (sensitivity 70%, specificity 71.4%, positive predicted value 70%, negative predicted value 71.4%, area under the curve 0.709, 95% confidence interval 0.59–0.83, and \( P = 0.001 \)) (Fig. 4).

**DISCUSSION**

IFI16, as a molecule encoded by IFN-inducible genes, is a phosphoprotein that is localized to the nucleus, especially in keratinocytes, epithelial cells, hematopoietic cells including lymphocytes and monocytes cells, and vascular endothelial cells [37,38].

It consists of C-terminal HIN domain and N-terminal PYD (pyrin) domain; the latter domain mediates its main proinflammatory effector function [39]. This protein acts as pattern recognition receptors in virally infected cells that can sense the presence of virally derived genetic materials [40,41,19] and functions as a restriction factor against viral replication [42]. In addition, it induces type I

**Table 4: Correlation between serum level of interferon-inducible protein-16 and anti-interferon-inducible protein-16 antibodies and studied variables among patients with rheumatoid arthritis**

<table>
<thead>
<tr>
<th>With</th>
<th>Serum IFI16 ((n=82))</th>
<th>Serum IFI16 Ab ((n=82))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.09 (P = 0.42)</td>
<td>0.082 (P = 0.46)</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>0.205 (P = 0.064)</td>
<td>0.05 (P = 0.66)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.09 (P = 0.69)</td>
<td>0.04 (P = 0.72)</td>
</tr>
<tr>
<td>DAS28</td>
<td>0.114 (P = 0.22)</td>
<td>0.107 (P = 0.33)</td>
</tr>
<tr>
<td>Anti-CCP titer (u/ml)</td>
<td>0.478 (&lt;0.001) (HS)</td>
<td>0.331 (0.037) (S)</td>
</tr>
<tr>
<td>RF titer (u/ml)</td>
<td>0.206 (P = 0.063)</td>
<td>0.022 (P = 0.84)</td>
</tr>
<tr>
<td>Larsen’s score</td>
<td>0.209 (P = 0.062)</td>
<td>0.01 (P = 0.93)</td>
</tr>
</tbody>
</table>

Ab, antibody; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, disease activity score with 28-joint count; HS, highly significant; IFI16, interferon-inducible protein-16; S, significant.

**Table 5: Correlation between synovial level of interferon-inducible protein-16 and anti-interferon-inducible protein-16 antibodies and studied variables among patients with rheumatoid arthritis**

<table>
<thead>
<tr>
<th>With</th>
<th>Synovial IFI16 ((n=36))</th>
<th>Synovial IFI16 Ab ((n=36))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.264 (P = 0.12)</td>
<td>0.046 (P = 0.79)</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>0.129 (P = 0.51)</td>
<td>0.115 (P = 0.66)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.273 (P = 0.107)</td>
<td>0.203 (P = 0.066)</td>
</tr>
<tr>
<td>DAS28</td>
<td>0.228 (P = 0.18)</td>
<td>0.235 (P = 0.17)</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>0.272 (P = 0.107)</td>
<td>0.247 (P = 0.13)</td>
</tr>
<tr>
<td>RF</td>
<td>0.147 (P = 0.39)</td>
<td>0.136 (P = 0.43)</td>
</tr>
<tr>
<td>Larsen score</td>
<td>0.149 (P = 0.38)</td>
<td>0.135 (P = 0.41)</td>
</tr>
</tbody>
</table>

Ab, antibody; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, disease activity score with 28-joint count; IFI16, interferon-inducible protein-16; RA, rheumatoid arthritis.

**Figure 2:** Plain HRCT of the chest of a 65-year-old female patient with RA (pulmonary window, axial cut) revealed multiple bilateral variable-sized peripherally based pulmonary nodules with peripheral honey-combing and fine reticulations (ground-glass opacities are clearly seen in other cuts). HRCT, high-resolution computed tomography; RA, rheumatoid arthritis.

**Figure 3:** Box plot for the median and IQR of IFI16 and anti-IFI16 antibodies titer in serum and synovial fluid among the studied groups. IGI16, interferon-inducible protein-16; IQR, interquartile range.

**Figure 4:** ROC curve for the performance of serum IFI16 and anti-IFI16 antibodies in prediction of patients with RA with pulmonary involvement. IFI16, interferon-inducible protein-16; RA, rheumatoid arthritis; ROC, receiver operating characteristic.
IFI16 can be mis-located into cytoplasm and released extracellularly in several inflammatory conditions and during viral infection [42,50].

Extracellular circulating IFI16 was reported to be released from patients with different autoimmune diseases and was accessible to autoantibody production and breakage of immune tolerance [50–54]. This marker was found to be related to RA-ILD [29].

So, the aim of the study was to detect serum and synovial level of IFI16 and anti-IFI16 antibodies titers in patients with RA, to confirm the relation to pulmonary involvement, and to find out the possible relation to a particular pattern of RA-ILD as well as the relation to other clinical parameters of the disease.

The present work confirms the findings of a previous report [29], as serum and synovial concentration of IFI16 and anti-IFI16 antibodies were significantly elevated than that of the controls, suggesting a conceivable role in RA.

IFI16 functions as an ‘alarmin’ that is released from one cell to affect the neighboring cell during stressful conditions such as oxidative stress [55], similar to high mobility group box 1 protein [50,56] and heat shock proteins [57,58]. Such molecules provide a signal for secretion of plethora of potent cytokines, especially tumor necrosis factor-α and IL6 [57].

The presence of IFI16 in synovial fluid as reported in the current work suggested that the inflamed synovium may be considered as a site for extracellular release of this protein and a poor correlation between serum and synovial concentration of IFI16, suggesting another site for extracellular liberation.

Owing to the role of endothelial cell as an active player in inflammation and autoimmune diseases, there is some evidence suggested that IFI16 is internalized by endothelial cells followed by activation of intracellular pattern recognition receptors [50].

Additionally, IFI16 performs several functions on endothelial cells, especially during the initial stages of autoimmune diseases. It induces expression of intercellular adhesion molecules, ICAM-1 and VCAM-1, as well as chemokines factors for neutrophils and monocytes, CCL20, CCL2, CCL5, RANTES, and IL8, in addition to augmentation of TLR4 expression; this action is mediated through several signaling molecules such as MAPK and NF-κB [50,59,60], suggesting proinflammatory characteristics of IFI16.

The role of TLR4 and TLR2 and more recently TLR5 and TLR7 in RA pathogenesis was reviewed in previous reports; they function through direct and indirect ways to facilitate inflammatory response, RA angiogenesis, and subsequently, joint destruction [61].

Besides the induction of TLR4 expression in endothelial cells, IFI16 acts as a trigger of other TLRs, so it functions like DAMPs molecules when it is translocated from its nuclear compartment to outside the cells and stimulates signaling pathways and proinflammatory cytokines production [62,63].

Regarding other diseases such as psoriasis, IFI16 was up-regulated in keratinocytes not only by double-stranded DNA but also by other cytokines such as IL22, IL17, IFNγ, and tumor necrosis factor-α [53]. So, these processes magnified a vicious cycle of activation.

In our results, we showed that there was a positive correlation between IFI16 and anti-IFI16 AB production in serum and in synovial fluid, and this is in agreement with other reports in psoriatic arthritis and Sjögren’s syndrome. In those studies, the epitope that is recognized by anti-IFI16 AB is outside the functional DAPIN domain, so it is left free for performing its effector function in inflammation, suggesting that the role of those antibodies may be pathogenic rather than protective. Additionally, anti-IFI16 AB were found to be correlated with inflammatory markers in psoriatic arthritis and with decreased salivary and tear secretion, as well as anti-nuclear antibody positivity and high focus score in Sjögren’s syndrome [52,64].

However, these reports differ from our results in which these markers were not related to disease activity but rather to pulmonary affection and other autoantibodies production.

This is confirmed by other reports that stated that the role of IFN signaling in RA established disease is not related directly to general disease activity but rather represents a different pathologic or immune response activation pattern when compared with patients without this signaling, and this will influence the response to therapy, particularly biologics [65,66].

Regarding RA-ILD, the most reported pattern of interstitial disease is UIP with lesser extent NSIP [67] (in our study, 26 patients presented with UIP vs. 11 patients presented with NSIP and three patients with mixed pattern). In NSIP, the predominant feature is inflammatory infiltrations into alveolar septum [68] that are mediated by Th1 cytokines [69], which turned to Th2 cytokines, IL13, IL4, and IL10 [70] as well Th17 cytokines when the fibrotic phase predominates [71].

In UIP, the main pathogenic player is activated myofibroblast with excessive extracellular matrix formation that progresses to lung fibrosis in the absence of prominent inflammation [72]. The concordance between RA-ILD and anti-CCP antibodies was reported in two recent Egyptian studies, in which autoantibodies positivity is associated with higher reticular and fibrotic scores, confirming the relation to UIP pattern [73,74].

In this work, patients with RA who presented with pulmonary interstitial disease had higher serum level of IFI16 and anti-IFI16 AB than other patients with RA. However, Alunno et al. [29] reported that RA pulmonary involvement is related to circulating level of IFI16 rather than anti-IFI16 antibodies.

By reviewing the relation between IFI16 and ILD, some works emphasized on the role of IFI16 in idiopathic pulmonary
fibrosis (IPF) by delineating the relation to viral infection such as cytomegalovirus, herpes virus [75,76], and Epstein–Barr virus [77].

Another work discovered ELMOD2 as a susceptibility gene involved in IPF. This gene is one of the larger group of genes that encode certain proteins containing engulfment and motility (ELMO) domain and participate in antiviral response. ELMOD2 induces several genes involved in the IFN-1 signaling, mostly IFI16 [78].

The previous observations underlined the role of IFI16 in IPF that share several characteristics with RA-ILD including demographic features such as male sex predominance and smoking [79] as well as radiological and pathological picture of UIP [67] and in addition to the role of citrullinated protein [80,81], and these may provide a clue for the relation between IFI16 and anti-CCP antibodies, as reported in our study.

Related to other ILDs, Benmerzoug et al. [82] stated that IFI16-induced STING pathway is necessary for development of silica-induced pulmonary disease, and this pathway is stimulated in response to self-DNA, which is released after airway cellular apoptosis induced by silica inhalation, and this process culminates in activation of NLRP3 inflammasome, CXCL 10, and interstitial inflammation with subsequent fibrosis.

So, IFI16 may relate to different diseases of lung fibrosis, and this study highlighted the possible role in RA-ILD. More recent research studies demonstrated that the mixed pattern of RA-ILD was observed in a significant number of patients [83–85], so, this may clarify the nonsignificant differences between UIP and other patterns regarding serum level of IFI16 protein and antibodies reported in our work, and this stage may represent an early phase of transition to a mixed pattern.

In this study, we found out the relation between anti-IFI16 antibodies and RA-ILD as well anti-CCP antibodies, in addition to the previously reported relation with IFI16 protein. So, anti-IFI16 antibodies could be considered as a novel marker for RA-ILD, but these findings need to be confirmed by a larger number of patients. This study did not define the predilection to certain pattern of ILD, and larger follow-up studies are needed to ratify these results.

CONCLUSION

IFI16 and anti-IFI16 antibodies were found to be elevated in serum and synovial fluid in patients with RA when compared with controls, and both markers were correlated with anti-CCP antibody production. No correlation was found to disease activity, joint erosions, or other serological markers. Patients who presented with pulmonary involvement showed higher level of those markers than patients who presented with other extra-articular manifestations, but no relation to certain types of pulmonary ILD was found. So, serum IFI16 and anti-IFI16 antibodies could be used as valuable indicators of this disease, but larger studies needed to confirm.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

The significance of IFI16 and anti-IFI16 in RA-ILD


