Positive anti-cyclic citrullinated proteins and Rheumatoid Factor during active lung Tuberculosis.

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**ABSTRACT**

**Background:** Tuberculosis (TB) infection, which involves a chronic stimulation of the immune system, may share similarities with connective tissue diseases, such as fever, myalgia, pulmonary and musculoskeletal involvement. Anti-cyclic citrullinated peptides (CCP) are considered as the most specific autoantibody in RA.

**Objectives:** To determine the prevalence of anti-CCP and IgM-RF in sera of patients with TB compared with healthy control subjects.

**Materials and Methods:** Consecutive patients with recently diagnosed active pulmonary TB (N=47, age 47±21, 29 males/21 females) and 39 healthy controls participated in this study. Data were collected on a questionnaire including clinical features of the disease, duration of symptoms, fever, cough, arthralgia, myalgia, sicca symptoms and others. Sera of the patients were collected before starting treatment for TB and frozen at -20°C. Anti-CCP and IgM-RF were evaluated by ELISA.

**Results:** The mean duration of TB related symptoms was 4.4±1.7 months, 73% had fever, 94% presented with cough. Rheumatic symptoms were relatively rare: arthralgia (4%), myalgias (4%), eye and mouth dryness (2 and 8%, respectively). The mean levels of anti-CCP were significantly increased in TB patients in comparison with controls: 44.9±51 IU vs. 20±7.3 IU (p=0.002). Serum levels above >40 U (moderately positive) were found in 32% of patients in comparison with 2.6% of controls (p=0.002). The mean serum levels of IgM-RF were significantly increased in TB patients: 17.8±19 vs. 4.3±5 (p<0.0001). IgM-RF was found positive (>6 IU) in 62% of patients in comparison with 2.6% of controls (p<0.0001).

**Conclusions:** A significant proportion of patients with active TB present increased titer of anti-CCP and IgM-RF. These findings should be considered in the interpretation of serological studies in patients with systemic manifestations.

Key words: anti-CCP IgM-RF Tuberculosis
**Introduction**

The last two decades have been marked by a worldwide resurgence of Tuberculosis (TB). Approximately 2 billion people have latent TB and about 8 million would develop active TB (1). Not only pulmonary but also cases of extra pulmonary TB have been shown to increase (2). Approximately 10% of extra pulmonary TB involves the bones and joints (2). Patients with TB, even without evidence for a direct musculoskeletal / local involvement, may present with a variety of rheumatic symptoms and signs. Although TB arthritis most commonly manifests as a monoarthritis of the weight-bearing joints (3), oligoarticular or polyarticular presentation is not rare, mimicking inflammatory diseases such as spondyloarthropathies (4) or / and Rheumatoid arthritis (RA) (5). Poncet’s disease is the classical example of tuberculosis-induced polyarthritis (6). Furthermore, the sera of patients with TB may contain Rheumatoid factors in up to 40% of the cases (7).

Antibodies recognizing cyclic citrullinated peptides are highly specific for RA (8). The specificity for RA has been shown to be up to 98% in comparison with 0-1 % of healthy controls and 2-5% of disease controls (8). Anti-CCP antibodies are present early in the disease process and may even pre-date the onset of RA by many years (9). The aim of this study was to determine the prevalence and associations of anti-CCP antibodies in patients with TB.

**Patients and Methods**

**Patients**

Forty-seven patients with recently diagnosed active pulmonary TB were included in the study. All were hospitalized in the TB Department of Tuberculosis, with clinical symptoms and radiological signs of TB as well as positive cultures for Mycobacterium tuberculosis. A special questionnaire was used with data on the clinical features of the disease, such as duration of symptoms, the presence of fever, cough, as well as rheumatological manifestations such as arthralgia/arthritis, myalgia, rash, mucocutaneous symptoms, sicca symptoms, spontaneous abortion, history of thrombosis and familial history of autoimmune diseases. Sera were collected before starting treatment for TB and frozen at -20ºC. Controls were 39 aged matched healthy personnel.

**Anti-CCP**

Serum IgG anti-CCP 2 was studied by ELISA using a commercial kit (Quanta lite™, Inova diagnostics, San Diego CA, USA), according to the Manufacter’s instructions. Samples were considered weakly positive if the antibody titer was 20-39 IU, moderately positive between 40 and 59 and strongly positive >60 IU.

**IgM Rheumatoid Factor.**

IgM rheumatoid factor serum was performed using an ELISA commercial kit (Quanta lite™, Inova diagnostics, San Diego CA, USA), according to the Manufacturer’s instructions. Samples were considered positive if the antibody titre was above 6 IU.
Statistical analysis
Statistical analysis was performed by the SPSS software, using Student’s t test and chi square to compare antibodies titers or positivity rate, respectively, between TB patients and controls. Pearson correlation coefficients were used to study the relationship between clinical parameters and the levels of anti-CCP and IgM-RF. P<0.05 was considered significant.

Results
Patients
Table 1 summarizes the demographic and clinical characteristics of TB patients and healthy controls. The patients had a mean duration of symptoms of 4.4±1.7 months; 73% had fever, 94% presented with cough. Only a small minority had symptoms such as arthralgia (4%), myalgias (4%), or eye and mouth dryness (2 and 8% respectively). None of the patients had signs typical of Rheumatoid Arthritis such as arthritis, morning stiffness or rheumatoid nodules. None had mucocutaneous aphtae or skin manifestations or a history of spontaneous abortion, thrombosis or known first-degree familial autoimmune disease.

Serum levels of anti-CCP and IgM-RF.
The mean levels of anti-CCP were significantly increased in TB patients in comparison with controls: 44.9±51 IU vs. 20±7.3 IU (p=0.002). Serum levels above the upper normal limits (>40 IU) were found in 32% of patients in comparison with 2.6% of controls (p=0.002). (Figure 1).
The mean serum levels of IgM-RF were significantly increased in TB patients: 17.8±19 vs. 4.3±5 (p<0.001). IgM- RF was found positive (> 6 IU) in 62% of patients in comparison with 2.6% of controls (p=0.000<0.01). (Figure 2).
In patients with increased levels of anti-CCP and IgM-RF, the mean levels were of 126.3±52 IU (range: 49.7-205) and 32.8±31.4 IU (range: 6.1-105) respectively.

Associations between clinical manifestations and serological studies
The presence of anti-CCP significantly correlated with a history of prolonged of fever (p=0.005). No correlation was found between the presence of anti-CCP or IgM-RF and any rheumatic symptom. A significant correlation was found between symptoms of fever and cough (p=0.003), and between arthralgia and sicca (p<0.0001).
No association was found between anti-CCP and RF.
Discussion

In the present study, we observed that 15 out of 47 (32%) of patients with TB have positive levels of anti-CCP. Although the presence of anti-CCP correlated with fever, it was not associated with symptoms and signs of arthritis. Antibodies to cyclic citrullinated peptides are a family of antibodies with specificities directed against a variety of citrullinated peptide (8). Antibodies to CCP are present in the majority of patients with RA and have been found to have a specificity of more than 90% (8). However, recent reports have shown the presence of anti-CCP in the serum of 8% of patients with Psoriatic Arthritis have questioned its specificity (10). It is not clear whether the false positive anti-CCP reactivity observed in TB patients is directed against citrullinated or not citrullinated epitopes in the substrate for the CCP test. Except for hepatitis C (HCV), the presence of anti-CCP in infectious diseases has not been well studied. It seems that in contrast to RF, which is present in a great majority of patients with hepatitis C arthritis, as well as in a proportion of patients with other subacute infections, notoriously bacterial endocarditis, anti-CCP is negative in HCV patients and may help in discriminating between HCV related arthritis and RA (11,12).

Tuberculosis is a multifaceted disease, which may present with a variety of symptoms, sometimes mimicking autoimmune diseases. Involvement of the bones and joints is the most frequent extra pulmonary manifestation of TB (2). There are many pitfalls in the diagnosis of tuberculous arthritis. Clinically, it may imitate other inflammatory rheumatic diseases, such as rheumatoid arthritis (5). The triad of radiologic abnormalities observed in tuberculous arthritis may simulate RA and includes periarticular osteoporosis, peripherally located osseous erosion and gradual diminution of the joint space (13). Furthermore, Rheumatoid factor has been reported in up to 40% of patients with TB (7). Djavad et al have analysed the rheumatoid factors produced by Epstein-Barr virus transformed monoclonal B cells obtained from patients with RA and TB, showing similarity in terms of avidity and affinity to different antigens (14). In addition to RF, TB patients may present a variety of autoantibodies (15) such as ANA, anti-cardiolipin (15) and anti-neutrophilic cytoplasmatic antibodies (16). Thus patients with TB may present both clinical and laboratory parameters with rheumatic conditions in general and RA in particular.

In conclusion, a significant proportion of our present series of patients with active TB presented higher titers of anti-CCP and IgM-RF. These findings should be kept in mind in the interpretation of serological studies in the differential diagnosis of patients with systemic manifestations.
REFERENCES

**Table 1**

<table>
<thead>
<tr>
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<th>Patients with TB (N=47)</th>
<th>Controls (N=39)</th>
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<tbody>
<tr>
<td>Age</td>
<td>52.3±17</td>
<td>47±21</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>29/21</td>
<td>13/26</td>
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<tr>
<td>Duration of symptoms (months)</td>
<td>4.4±1.7</td>
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<tr>
<td>No (%) of patients with:</td>
<td></td>
<td></td>
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<tr>
<td>Fever</td>
<td>34 (73)</td>
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<tr>
<td>Cough</td>
<td>42 (94)</td>
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<tr>
<td>Arthralgia</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>Myalgia</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>Eye dryness</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Mouth dryness</td>
<td>4 (8)</td>
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</tbody>
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Serum IgM-RF in TB patients and Controls
Serum anti-CCP in TB patients and Controls