Autoantibody prevalence in active tuberculosis: reactive or pathognomonic?

Chieh-Yu Shen, Song-Chou Hsieh, Chia-Li Yu, Jann-Yuan Wang, Li-Na Lee, Chong-Jen Yu

ABSTRACT

Objectives: To evaluate the autoantibody in patients without corresponding symptoms, whether these autoantibodies are pathognomonic or not. We hypothesised that autoantibody may be reactive to chronic infection, such as tuberculosis (TB).

Design: Randomised, case–control cohort study.

Setting: A tertiary centre in Taiwan.

Participants: We randomly chose 100 patients out of the data bank of patients with TB in a tertiary medical centre. All patients completed the sera sampling.

We chose 100 patients according to autoantibody prevalence in previous literature. We also chose 100 medical staff as control group.

Interventions: We tested anti-SSA, anti-SSB, anti-Sm, anti ribonucleoprotein, anti-Scl 70, anticientromere, anti-double-stranded DNA, anticardiolipin IgG and IgM in all patient and control groups. The clinical symptoms and the underlying disease were all recorded.

Primary and secondary outcome measures: The result of sera antibody titre was recorded. For those with specific positive serology results, following examination was carried out after a 3-month anti-TB medication.

Results: Anticardiolipin IgG titre was significantly higher in patients with TB than in control group. We compared the result with previous population study and found that anti-Scl70 is also significantly higher in patients with TB. The following up data in anti-Scl70 revealed decreased titre after treatment. No correlation between sera titre and clinical conditions was observed.

Conclusions: In TB endemic areas, a significant proportion (32%) of patients with TB have elevated autoantibody titres, especially anticardiolipin IgG and anti-Scl-70. Mycobacterial studies should be performed in patients with elevated serum autoantibody titres but without the typical or multiple manifestations of autoimmune diseases.

Trial registration: The study was approved by the Institutional Review Board of the hospital (NTUH REC: 9561707008) after informed consent had been obtained from the patients.

INTRODUCTION

Tuberculosis (TB) has become one of the most important diseases in the past two decades. It leads to organ dysfunction, mortality and various clinical manifestations. Previous studies have shown that sera from patients with active TB may contain autoantibodies that are unique in autoimmune diseases. The reported autoantibodies include rheumatoid factor (RF), antinuclear antibody (ANA), anticardiolipin antibody (ACA; IgM isotype predominant), antineutrophil cytoplasmic antibodies (ANCA) and anticyclic citrullinated peptide. Some of these are hallmarks of certain autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus (SLE) and ANCA-associated vasculitis. Some are even disease-specific, such as anticyclic citrullinated peptide in rheumatoid arthritis. However, most studies are cross-sectional with limited case numbers and without definite correlations between...
immune interaction between chronic active infection and autoimmunity

serology and clinical manifestations. Little is known on the clinical significance (ie, pathognomonic, reactive or incidental) of autoantibodies in TB and the necessity of corticosteroid therapy.

Since patients with TB present with non-specific symptoms like fever, malaise and weight loss, clinicians may simultaneously order mycobacterial studies (acid-fast smear and mycobacterial culture) and autoimmune serology. Results of the latter usually become available earlier than results of the former. Thus, some patients with TB are put on systemic corticosteroids rather than anti-TB treatment, rendering the further dissemination of Mycobacterium tuberculosis bacilli. In this prospective cohort study, the prevalence of autoantibodies in patients with active TB was evaluated and compared with those of healthy controls. Dynamic changes in the autoantibodies were also monitored to investigate their clinical significance in patients with TB.

PATIENTS AND METHODS

Patients and the study protocol

The Institutional Review Board of the National Taiwan University Hospital (NTUH) approved this study (NTUH REC: 9561707008). To have a power of 0.8 and an α error of 0.05 in a two-sided test where the prevalence of ANA in patients with TB and the general population was 33% and 20%, respectively, the calculated sample size was 83 for each. Therefore, from the 933 new cases of culture-confirmed TB, diagnosed at the NTUH between January 2007 and December 2009, 100 were enrolled. All of the study participants provided written informed consent.

Among the 100 patients with TB, 96 had pure pulmonary TB, two had concomitant pulmonary and extrapulmonary TB (peritonitis in one and meningitis in another) and two had extrapulmonary TB only (neck lymphadenopathy in one and cutaneous TB in another). The first serum samples were collected before the start of anti-TB treatment. Blood was examined for autoantibodies to the Ro antigen, La antigen, centromere protein, double-stranded DNA (dsDNA), ScI-70, Smith protein, RNP, histone protein and Jo1. Serum samples were prepared at 1:21 dilution and ELISA was performed according to the manufacturer’s instructions.

A commercially available kit QUANTA Lite ACA IgM III was used to test anticardiolipin IgM. Sera were prepared at 1:101 dilution. The commercial kit Phadia Varelisa Cardiolipin IgG Antibodies EIA kit was used to test anticardiolipin IgG at serum dilution of 1:101. All ELISA assays were performed according to the manufacturers’ instructions.

Detection of autoantibodies

A commercial test system AtheNA Multi-Lyte ANA-II Plus Test System was used to test IgG class anti-extractable nuclear antigens, including autoantibodies to the Ro antigen, La antigen, centromere protein, dsDNA, ScI-70, Smith protein, RNP, histone protein and Jo1. Serum samples were prepared at 1:21 dilution and ELISA was performed according to the manufacturer’s instructions.

Statistical analysis

Intergroup difference was calculated using independent-samples t test for continuous variables and χ² test or Fisher’s exact test for categorical variables, as appropriate. Statistical significance was set at p<0.05. All analyses were performed using the SPSS software V12.0 for Windows.

RESULTS

The clinical characteristics of the 100 patients with TB were summarised in table 1. The median age was 63 years (range, 19–92 years), with a male-to-female ratio of 2.03. Forty-one patients with TB had underlying diseases such as malignancy (n=17), diabetes mellitus (n=14), end-stage renal disease (n=9) and one each with SLE, anklylosing spondylitis and rheumatoid arthritis.
Among the 17 patients with TB with malignancies, 6 had lung cancer, 3 had haematological malignancies, 4 had airway and lung malignancies, 2 had prostate cancer, 1 had breast cancer and 1 had pancreatic cancer.

The SLE patient was a middle-aged woman who presented as polyarthritis, malar rash and positive anti-dsDNA and antinuclear antibodies. She received disease-modifying antirheumatic drugs (DMARDs) and corticosteroids. The patient with ankylosing spondylitis was a young woman diagnosed by clinical symptoms and positive HLA-B27. She received non-steroidal anti-inflammatory drugs (NSAIDs). The patient with rheumatoid arthritis was a young woman with symmetric polyarthritis with positive RF. She received DMARDs and NSAIDs. Before the diagnosis of pulmonary TB, results of autoantibody tests were all negative in these three patients.

Thirty-two patients with TB had elevated serum autoantibody levels, including nine with more than one autoantibody (table 1). The clinical manifestations and radiographic findings of the autoantibody-positive and antibody-negative groups were similar. Cough and sputum production were the most common respiratory symptoms, while fever and weight loss were the most common constitutional symptoms. Thirty-one patients were sputum smear positive for acid-fast bacilli.

Detailed results of the autoantibody tests were shown in table 2. The most prevalent autoantibodies in patients with TB were anti-Scl-70, antihistone and anticardiolipin IgG. A significantly higher proportion of patients with TB had elevated serum anticardiolipin IgG titres than the healthy controls (p<0.001). Among the anti-Scl-70-positive and anti-Scl-70-negative groups, 16% and 9%, respectively, had pulmonary cavitation (p=0.441) and 33% and 16%, respectively, had pleural effusion (p=0.245).

The median age of the healthy controls was 30 years, with a male-to-female ratio of 0.33. None had underlying diseases. Though not statistically significant, the control group had a higher prevalence of anticardiolipin IgM than the TB group (table 1). Of the 10 healthy controls positive for anticardiolipin IgM, 8 had borderline titres that just passed the cut-off value. Among them, one was positive for anti-RNP and another for antihistone antibodies.

Within 3 months of anti-TB treatment, 61 patients had 148 adverse events (table 3). The most common were rheumatological events, followed by gastrointestinal events. There was no significant difference in adverse events between patients with autoantibody-positive and autoantibody-negative TB.

For the 11 patients with elevated anticardiolipin IgG and 6 patients with elevated anti-Scl-70 at baseline, serum titres were followed up after 3 months of anti-TB treatment (table 4). None of the patients received immunosuppressants and DMARDs. Follow-up serum titres of anticardiolipin IgG and anti-Scl-70 returned to normal limits in seven and four patients, respectively. Among the five patients with persistently elevated anticardiolipin IgG or anti-Scl-70 autoantibodies, none had rheumatological symptoms at the end of the 6-month anti-TB treatment.

<table>
<thead>
<tr>
<th>Table 1 Clinical characteristics of patients with autoantibody-positive and autoantibody-negative tuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autoantibody-positive (n=32)</strong></td>
</tr>
<tr>
<td>Respiratory symptoms</td>
</tr>
<tr>
<td>Cough</td>
</tr>
<tr>
<td>Sputum</td>
</tr>
<tr>
<td>Haemoptysis</td>
</tr>
<tr>
<td>Dyspnoea</td>
</tr>
<tr>
<td>Chest pain</td>
</tr>
<tr>
<td>Constitutional symptoms</td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Weight loss</td>
</tr>
<tr>
<td>General malaise</td>
</tr>
<tr>
<td>Night sweating</td>
</tr>
<tr>
<td>Sputum smear grading</td>
</tr>
<tr>
<td>3+~4+</td>
</tr>
<tr>
<td>1+~2+</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Serum albumin &lt;3.5 g/dL</td>
</tr>
<tr>
<td>Radiographic findings</td>
</tr>
<tr>
<td>Bilateral infiltration</td>
</tr>
<tr>
<td>Cavitations</td>
</tr>
<tr>
<td>Pleural effusion</td>
</tr>
<tr>
<td>Miliary lesion</td>
</tr>
</tbody>
</table>

Data are number (%).
DISCUSSION

The present study has three important findings. First, one-third of patients with active TB had elevated serum autoantibodies. The prevalences of their autoantibodies, especially anticardiolipin IgG and anti-Scl-70, were significantly higher than those of the general population. Second, consistent with previous studies, the presence of autoantibodies neither altered the clinical manifestations and radiographic findings of active TB nor changed the risk of developing adverse events during anti-TB treatment. Third, the elevated autoantibody levels returned to normal limits simply by anti-TB treatment and not by immunosuppressive therapy. These findings suggest that increased serum autoantibodies during active TB may not be diagnostic of autoimmune diseases. Clinical correlation and follow-up are still necessary.

Autoantibodies come from a break in self-tolerance whereby fragments of mixed self-antigens and pathogen antigens may induce immune response, as in a mode of epitope spread and autoantibody production. Epitope spread occurs when there is chronic inflammation, causing the immune system to produce a variety of antibodies against pathogens of chronic infections. Active TB is one of the most common infectious diseases causing long-term inflammation and tissue destruction. Thus, it is reasonable that autoantibodies are more common in patients with TB.

Clinically detectable autoantibodies are often characteristic of certain autoimmune diseases. However, like in other clinical examinations, all autoantibodies should be tested based on the corresponding clinical symptoms and signs. Most autoantibodies are pathognomonic of certain conditions, such as anti-dsDNA in SLE, anticyclic citrullinated peptide antibody in rheumatoid arthritis, anti-Scl-70 in systemic sclerosis and anti-Jo-1 in polymyositis. However, autoantibodies may also be present in conditions other than autoimmune diseases, especially when there are no corresponding clinical symptoms, such as RF in infective endocarditis and antiphospholipid syndrome secondary to infection or malignancy.

The high prevalence of serum autoantibodies in active patients with TB has rarely been investigated and thus, remains unclear. If the autoantibodies are

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>ELISA-pos</th>
<th>Percentage of pos</th>
<th>Patients with TB (n=100)</th>
<th>Healthy control (n=100)</th>
<th>p Value</th>
<th>TB vs normal</th>
<th>TB vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Ro</td>
<td>58</td>
<td>2.7</td>
<td>2</td>
<td>2</td>
<td>0.855</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anti-La</td>
<td>5</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0.795</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anti-RNP</td>
<td>11</td>
<td>0.5</td>
<td>2</td>
<td>1</td>
<td>0.138</td>
<td>0.108</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Scl-70</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.498</td>
</tr>
<tr>
<td>Anti-Jo1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>&lt;0.001</td>
<td>0.044</td>
<td>1</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>10</td>
<td>0.5</td>
<td>1</td>
<td>0</td>
<td>0.579</td>
<td>0.39</td>
<td>1</td>
</tr>
<tr>
<td>Anticentromere</td>
<td>30</td>
<td>1.4</td>
<td>5</td>
<td>2</td>
<td>0.101</td>
<td>0.059</td>
<td>0.683</td>
</tr>
<tr>
<td>Antithistone</td>
<td>NA</td>
<td></td>
<td>11</td>
<td>7</td>
<td></td>
<td></td>
<td>0.323</td>
</tr>
<tr>
<td>Anticardiolipin IgM</td>
<td>NA</td>
<td></td>
<td>6</td>
<td>10</td>
<td></td>
<td></td>
<td>0.297</td>
</tr>
<tr>
<td>Anticardiolipin IgG</td>
<td>NA</td>
<td></td>
<td>11</td>
<td>0</td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>

Adverse events during anti-tuberculosis (TB) treatment in patients with autoantibody-positive and antibody-negative TB

<table>
<thead>
<tr>
<th>Autoantibody-positive (n=32)</th>
<th>Autoantibody-negative (n=68)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatological events</td>
<td>10 (31)</td>
<td>28 (41)</td>
</tr>
<tr>
<td>Gastrointestinal events</td>
<td>9 (28)</td>
<td>22 (32)</td>
</tr>
<tr>
<td>Neurological events</td>
<td>8 (25)</td>
<td>16 (24)</td>
</tr>
<tr>
<td>Renal events</td>
<td>7 (22)</td>
<td>19 (28)</td>
</tr>
<tr>
<td>Constitutional events</td>
<td>4 (13)</td>
<td>19 (28)</td>
</tr>
<tr>
<td>Respiratory events</td>
<td>2 (6)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Haematological events</td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Total number of events</td>
<td>41</td>
<td>107</td>
</tr>
</tbody>
</table>

Data are number (%). TB, tuberculosis.
pathognomonic, there should be corresponding rheum- 
atological symptoms and signs, especially in those with specific antiextractable nuclear antigen antibodies. Since the presence of autoantibodies does not alter the clinical manifestations and radiographic presentations of active TB, it is unlikely that autoantibodies have a path-
gnomonic role. In autoimmune diseases, serology 
titres of autoantibodies often change along with disease activity, such as anti-dsDNA in lupus nephritis. However, the finding that autoantibody titres return to normal limits after anti-TB treatment in more than two-thirds of patients with TB suggests the opposite— that the increase in autoantibodies is a reactive change during the disease course of active TB. This is a very important reminder.

If a patient has elevated autoantibody levels but no typical or multiple rheumatological symptoms, other possible aetiologies should be considered. For a patient in TB endemic areas, sputum samples for acid-fast smear and mycobacterial culture should be performed. If TB is missed and patients are diagnosed as having autoimmune diseases and treated with systemic corticoster-
oids, disseminated TB and TB-related mortality may occur.

The association of active TB and antiphospholipid syndrome, which is known to be secondary to infectious diseases. Such antibody may be triggered by an infectious process that is non-β2-glycoprotein 1-dependent. In active TB, mycobacterial phenolic glyco-
lipids may activate neutrophils and release containers of ectosomes. Vesicles released from activated neutrophils that express phosphatidylserine and annexin V may be the epitopes of antiphospholipid antibodies. This may explain the high prevalence of antiphospholipid IgG in patients with TB. In general, the transient positivity of antiphospholipid antibody is not considered path-
gnomonic and does not meet the diagnostic criteria of antiphospholipid syndrome.

However, some studies have different findings and show that patients with TB with diffuse alveolar haemor-
rhage have transient anticardiolipin IgG positivity. Moreover, in the present study, anticardiolipin IgG remained elevated in 4 of 11 patients even after 3 months of anti-TB treatment. Although anticardiolipin IgG is not diagnostic, a high index of suspicion should be maintained regarding their clinical manifestations, including thrombosis, haemorrhage, haemolytic anaemia and thrombocytopenia in patients with TB with elevated anticardiolipin IgG.

Anti-Scl-70 is the first known prevalent autoantibody in patients with progressive systemic sclerosis. The autoanti-
bodystargets an antigen that is a 70 kDa protein. Later studies reveal that Scl-70 should be the nuclear DNA body targets an antigen that is a 70 kDa protein. Later studies reveal that Scl-70 should be the nuclear DNA target.27,28 Topoisomerase I is an enzyme that relaxes the strain on DNA by nicking and ligating it. One of the notorious manifestations of systemic sclerosis is interstitial lung disease, characterised by lung fibrosis. Although there is no significant difference, patients with TB in this study have elevated anti-Scl 70 and higher risk of pulmonary cavitation and pleural effusion. The underlying mechanisms involved are unclear since antigenic topoisomerase I is not a component of leuko-
cyte ectosome. Moreover, it is not released from the TB bacilli because the structure of mycobacterial topo-
isomerase I is different from those of humans.

In the present study, anti-Scl-70 titres returned to normal range after anti-TB treatment in all except one patient. The anti-Scl-70 antibody in active TB is probably secondary to pulmonary inflammation and destruction, which is uncovered by the nuclear topoisomerase I, thereby triggering the production of autoimmunity. The titre decreases once pulmonary injury is alleviated.

This study has some limitations. First, because the control group was composed of medical staff members, their baseline characteristics were very different from those of the TB group. This might lead to uncertainty in the prevalence of autoantibody in non-TB groups. Nonetheless, the autoantibody prevalence was still higher.

### Table 4: Serum autoantibody titres in patients with TB before and after anti-TB treatment

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Anti-Scl-70 Initial</th>
<th>Anti-Scl-70 Follow-up</th>
<th>ACA-IgG Initial</th>
<th>ACA-IgG Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>137</td>
<td>155</td>
<td>18</td>
<td>18.1</td>
</tr>
<tr>
<td>18</td>
<td>156</td>
<td>35</td>
<td>36</td>
<td>12.3</td>
</tr>
<tr>
<td>51</td>
<td>125</td>
<td>28</td>
<td>55</td>
<td>10.8</td>
</tr>
<tr>
<td>64</td>
<td>152</td>
<td>55</td>
<td>62</td>
<td>31.1</td>
</tr>
<tr>
<td>65</td>
<td>154</td>
<td>79</td>
<td>81</td>
<td>14.6</td>
</tr>
<tr>
<td>91</td>
<td>153</td>
<td>68</td>
<td>94</td>
<td>12.8</td>
</tr>
</tbody>
</table>

Normal reference values for anti-Scl-70 and ACA-IgG are <100 U/mL and <10 GPL, respectively.

ACA, anti-cardiolipin antibody; TB, tuberculosis.
Immune interaction between chronic active infection and autoimmunity

in patients with TB than in the general population reported in a previous publication.10 Second, follow-up samples were only obtained in those with elevated serum levels of autoantibodies before anti-TB treatment. Dynamic changes in autoantibodies might have been missed.

TB has a kaleidoscope of presentations that constantly challenge physicians. In TB endemic areas, a significant proportion (32%) of patients with TB has elevated autoantibody titres, especially anticardiolipin IgG and anti-Scl-70. This phenomenon is likely to be reactive due to the lack of clinical correlations, as well as the spontaneous regression after TB treatment. Mycobacterial studies should be performed in patients with elevated serum autoantibody titres but without the typical or multiple manifestations of autoimmune diseases.

Acknowledgements The authors thank the staff of the Sixth and Eighth Core Labs, Department of Medical Research, and the Internal Medicine Lab of Immunology, National Taiwan University Hospital for their technical support.

Contributors J-YW and S-CH designed the study. C-Y, C-LY, S-CH, J-YW and L-NL all contributed to the manuscript writing and data interpretation. C-Y and J-YW were involved in the statistical analysis. C-JY was directly responsible for the general organisation and instruction. All authors have read and approved the final manuscript.

Funding This study was supported by the National Science Council, Taiwan (grant NSC 101-2322-B-002-008) and the Research Centre for Biotechnology and Medicine Policy (grant DOH-101-DG-1101). The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Competing interests None.

Ethics approval The study was approved by the Institutional Review Board of the hospital (NTUUHREC: 9561707008).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/3.0/

REFERENCES

Autoantibody prevalence in active tuberculosis: reactive or pathognomonic?

Chieh-Yu Shen, Song-Chou Hsieh, Chia-Li Yu, Jann-Yuan Wang, Li-Na Lee and Chong-Jen Yu

BMJ Open 2013 3:
doi: 10.1136/bmjopen-2013-002665

References
This article cites 25 articles, 3 of which you can access for free at:
http://bmjopen.bmj.com/content/3/7/e002665#BIBL

Open Access
This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/3.0/

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Immunology (including allergy) (57)
- Infectious diseases (548)
- Rheumatology (158)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/