New diagnostic approach for ocular tuberculosis by ELISA using the cord factor as antigen

Jun-ichi Sakai, Suguru Matsuzawa, Masahiko Usui, Ikuya Yano

Abstract

**Background and aims**—Diagnosis of ocular tuberculosis is difficult, particularly the retinal vasculitis type, because most cases occur without concurrent active pulmonary tuberculosis. Recently, it has been reported that detection of antibodies against purified cord factor (trehalose-6,6′-dimycolate, TDM), the best studied, most antigenic, and most abundant cell wall component of tubercle bacilli, is very useful for rapid serodiagnosis of pulmonary tuberculosis. In this study, an attempt was made to evaluate whether the detection of anticord factor antibody is also useful for diagnosis of ocular tuberculosis and the necessity of antituberculous therapy for tuberculosis retinochoroiditis was discussed.

**Methods**—Cases consisted of 15 patients with uveitis and retinal vasculitis, nine patients with presumed ocular tuberculosis, three patients with sarcoidosis, and three patients with Behçet’s disease. IgG antibodies against purified cord factor prepared from *Mycobacterium tuberculosis* H37Rv were detected by enzyme linked immunosorbent assay.

**Results**—All cases of clinically presumed ocular tuberculosis were positive, whereas all of the cases of sarcoidosis or Behçet’s disease were negative for anticord factor antibodies. When the anticord factor antibody titres were compared on the basis of the presence or absence of previous antituberculosis chemotherapy, the mean anticord factor antibody titre of the untreated group showed a tendency to be higher than in the treated group, but not significantly (p=0.07).

**Conclusions**—The detection of anticord factor antibody may be useful to support the diagnosis of ocular tuberculosis. Additionally, a positive result for anticord factor antibody may indicate that tubercle bacilli are present in some organ(s) of the patient even in the absence of active systemic disease.

**Materials and methods**

**SUBJECTS AND SERUM SPECIMENS**

Fifteen patients with uveitis and retinal vasculitis who visited the uveitis clinic of Tokyo Medical University Hospital in 1998 and 1999 were registered in this study. Nine patients with presumed ocular tuberculosis, three patients with sarcoidosis, and three patients with Behçet’s disease were enrolled; the characteristics of each patient are summarised in Table 1. Diagnoses of sarcoidosis and Behçet’s disease were based on the criteria of the disease research committee.7 A presumption of ocular tuberculosis was made on the basis of the presence of characteristic ocular lesions in the context of evidence of previous exposure to *Mycobacterium tuberculosis* (all cases were PPD skin test positive). All patients diagnosed as presumed ocular tuberculosis presented with retinal vasculitis, characterised by a relatively
Table 1  Patient baseline characteristics and anticord factor antibody titres

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Anterior uveitis</th>
<th>Retinal capillary non-perfusion</th>
<th>Retinal vasculitis</th>
<th>PPD skin test</th>
<th>Chest x ray</th>
<th>Clinical diagnosis</th>
<th>Titr (AI)</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>Presumed ocular TB</td>
<td>0.430</td>
<td>positive</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>Presumed ocular TB</td>
<td>0.700</td>
<td>positive</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>healed pulmonary TB</td>
<td>0.210</td>
<td>positive</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>healed pulmonary TB</td>
<td>0.150</td>
<td>positive</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>pulmonary TB</td>
<td>2.212</td>
<td>positive</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>Presumed ocular TB</td>
<td>0.258</td>
<td>positive</td>
</tr>
<tr>
<td>7</td>
<td>62</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>Presumed ocular TB</td>
<td>0.263</td>
<td>positive</td>
</tr>
<tr>
<td>8</td>
<td>67</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>healed pulmonary TB</td>
<td>2.469</td>
<td>positive</td>
</tr>
<tr>
<td>9</td>
<td>74</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>healed pulmonary TB</td>
<td>0.929</td>
<td>positive</td>
</tr>
<tr>
<td>10</td>
<td>54</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>BHL</td>
<td>0.100</td>
<td>positive</td>
</tr>
<tr>
<td>11</td>
<td>64</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>BHL</td>
<td>0.097</td>
<td>negative</td>
</tr>
<tr>
<td>12</td>
<td>45</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>BHL</td>
<td>0.013</td>
<td>negative</td>
</tr>
<tr>
<td>13</td>
<td>36</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>Behçet's disease</td>
<td>0.050</td>
<td>negative</td>
</tr>
<tr>
<td>14</td>
<td>34</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>Behçet’s disease</td>
<td>0.070</td>
<td>negative</td>
</tr>
<tr>
<td>15</td>
<td>49</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>Behçet’s disease</td>
<td>0.081</td>
<td>negative</td>
</tr>
</tbody>
</table>

*Positive: AI≥0.100.

mild degree of cellular infiltrate in the anterior chamber and vitreous, periphlebitis, and a marked tendency to peripheral retinal capillary non-perfusion leading to new vessel formation. Of the nine patients with presumed ocular tuberculosis, only one case had active pulmonary tuberculosis, four appeared to have old pulmonary tuberculosis on chest x ray films, and the others had no systemic disease. At the time when serum samples were obtained, cases 1, 3, and 4 already had a history of antituberculous therapy for suspected ocular tuberculosis (Table 2). Serum samples were obtained after obtaining informed consent from the patients.

ELISA
Cord factor was extracted and purified from a culture of M tuberculosis H37Rv, as reported previously. The purified cord factor antigen was dissolved in n-hexane at a concentration of 0.1 mg/ml, 25 µl of which was placed in each well of microtitre plates. A serum sample diluted 1:160 was added to each well after blocking with 0.05% Tween-20 in phosphate buffered saline (PBS-T). Goat antihuman immunoglobulin G labelled with peroxidase and diluted 1:160 was added to each well of microtitre plates. A serum sample diluted 1:500 with PBS-T was used as the second antibody. The absorption was read with a microplate reader at 492–630 nm.

DATA ANALYSIS
Anticord factor antibody titres were expressed as the absorption difference (AI)—that is, the difference in the absorption between the test serum and the same serum in wells not coated with antigen.

In our previous study, it was decided to record an AI equal to or above 0.100 as positive. That is, the mean AI of 100 healthy controls, among whom one person was tuberculosis negative and the others were all positive, was 0.016 (the absorption values of healthy controls was distributed between −0.102 and 0.099).

Statistical analysis of data was performed using the Mann–Whitney U test.

Results
Table 1 shows the ELISA results for the patients. When the cutoff point of AI was set at 0.100, the nine patients who were given a clinical diagnosis of presumed ocular tuberculosis were all positive. The three patients with sarcoidosis and three patients with Behçet’s disease were all negative. In the presumed ocular tuberculosis group, only one case (case 5) had active pulmonary disease, while the other eight had no active systemic disease. The case with active pulmonary disease (case 5) and three of the cases without evidence of active systemic disease (cases 2, 8, and 9) each had a high titre of anticord factor antibody.

When the anticord factor antibody titres were compared on the basis of the presence or absence of previous antituberculosis chemotherapy (Table 2), the mean anticord factor antibody titre of the untreated group (cases 2 and 5–9) showed a tendency to be higher than the mean of the treated group (cases 1, 3, and 4), but not significantly (p=0.07, estimated power 0.46).

The anticord factor antibody titres bore no relation to the length of the period from the onset of subjective symptoms until the assay for anticord factor antibody (Table 2).

Table 2  Treatment and outcome in presumed ocular TB

<table>
<thead>
<tr>
<th>Patient No</th>
<th>A</th>
<th>B</th>
<th>Antituberculous therapy</th>
<th>Laser photocoagulation</th>
<th>Vitrectomy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9 months</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>resolved</td>
</tr>
<tr>
<td>2</td>
<td>1 year over</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>inactive</td>
</tr>
<tr>
<td>3</td>
<td>1 year over</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>inactive</td>
</tr>
<tr>
<td>4</td>
<td>1 year</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>inactive</td>
</tr>
<tr>
<td>5</td>
<td>2 months</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>resolved</td>
</tr>
<tr>
<td>6</td>
<td>2 weeks</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>inactive</td>
</tr>
<tr>
<td>7</td>
<td>2 months</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>inactive</td>
</tr>
<tr>
<td>8</td>
<td>3 months</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>inactive</td>
</tr>
<tr>
<td>9</td>
<td>1 year</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>inactive</td>
</tr>
</tbody>
</table>

A = period from the onset of symptoms until the assay for anticord factor antibody
B = presence or absence of antituberculous therapy before the detection of anticord factor antibody.

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Discussion

As of several years ago it was generally accepted that the implication of tuberculosis as a cause of ocular disease had declined owing to the diminishing incidence of tuberculous infection and advances in the treatment of tuberculosis. However, today much attention is again being paid to tuberculosis as a cause of ocular disease had declined owing to the diminution of the participation of this bacterium as the antigen gave satisfactory results in confirming the diagnosis of ocular tuberculosis. That is, all cases of clinically presumed ocular tuberculosis were positive, whereas all cases of sarcoidosis or Behçet’s disease were negative. In spite of their common features of uveitis with retinal vasculitis, a clear-cut difference was thus ascertained between ocular tuberculosis and the other diseases.

All cases meeting our criteria—that is, suggestive of relatively typical ocular tuberculosis and consistent with published reports of retinal vasculitis associated with tuberculosis, and in which no other entity was suggested, were consecutively enrolled in this study. Thus we attempted to eliminate selection bias.

Maekura et al. reported that the antibody titre of patients with active pulmonary tuberculosis tended to decrease because of antituberculosis chemotherapeutic regimen. Consequently, a positive result for antituberculosis antibody titre may indicate that tuberculosis bacilli are present in some organ(s) of the patient, and that institution of antituberculosis chemotherapy must be considered. In our study, the mean titre of cord factor antibody of the untreated group was higher than that of the treated group, and antituberculosis chemotherapeutic regimen had resulted in a good prognosis for the presumed ocular tuberculosis.

In conclusion, the detection of antituberculosis antibody was very useful for supporting the diagnosis of ocular tuberculosis. Additionally, a positive result for antituberculosis antibody may indicate that tuberculosis bacilli are present in some organ(s) of the patient even in the absence of active systemic disease and possibly stimulate the immune system as an antigen in the pathogenesis of retinal vasculitis.

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