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

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

Abstract

Background/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 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. 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 vasculitis</th>
<th>Retinal capillary non-perfusion</th>
<th>PPD skin test</th>
<th>Chest x ray</th>
<th>Clinical diagnosis</th>
<th>Titre (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>normal</td>
<td>Presumed ocular TB</td>
<td>0.430</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>Presumed ocular TB</td>
<td>0.700</td>
<td>positive</td>
<td></td>
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<tr>
<td>3</td>
<td>68</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>healed pulmonary TB</td>
<td>Presumed ocular TB</td>
<td>0.150</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>healed pulmonary TB</td>
<td>Presumed ocular TB</td>
<td>0.210</td>
<td>positive</td>
<td></td>
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<tr>
<td>5</td>
<td>29</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>pulmonary TB</td>
<td>Presumed ocular TB</td>
<td>2.212</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Presumed ocular TB</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>62</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>normal</td>
<td>Presumed ocular TB</td>
<td>0.263</td>
<td>positive</td>
<td></td>
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<tr>
<td>8</td>
<td>67</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>healed pulmonary TB</td>
<td>Presumed ocular TB</td>
<td>2.469</td>
<td>positive</td>
<td></td>
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<tr>
<td>9</td>
<td>74</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>healed pulmonary TB</td>
<td>Presumed ocular TB</td>
<td>0.929</td>
<td>positive</td>
<td></td>
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<tr>
<td>10</td>
<td>54</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>BHL</td>
<td>Sarcoïdosis</td>
<td>0.098</td>
<td>negative</td>
</tr>
<tr>
<td>11</td>
<td>64</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>BHL</td>
<td>Sarcoïdosis</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>BHL</td>
<td>Sarcoïdosis</td>
<td>0.013</td>
<td>negative</td>
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<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>
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<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 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 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 tuberculin 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).
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 re-emerging infectious disease in association with an increase in the number of immunocompromised hosts and disregarded care for tuberculosis.5 Moreover, the acquisition of ocular fluid is useful for rapid serodiagnosis of pulmonary tuberculosis,6 and a new diagnostic approach for tuberculosis is generally accepted,291 6 yet the precise nature of that relationship has not been established. Though the question is unresolved as to whether the retinal vasculitic lesions contain active tubercle bacilli or reflect a hypersensitive response, we surmise that systemic antitubercular medication should be given to most patients with presumed ocular tuberculosis.

In conclusion, the detection of anticord factor antibody was very useful for supporting 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, and that institution of antituberculosis chemotherapy must be considered. In our study, the mean anticord factor antibody titre of the untreated group was higher than that of the treated group, and antituberculosis chemotherapy had resulted in a good prognosis for the presumed ocular tuberculosis (ischaemic retinal periphlebitis) (Table 2). A relation between retinal vasculitis and tuberculosis is generally accepted,4 5 15 yet we cannot deny the possibility that determination of the anticord factor antibody titre is useful for deciding therapeutic strategy. Consequently, a positive result for anticord factor antibody may indicate that tubercle bacilli are present in some organ(s) of the patient, and that institution of antituberculosis chemotherapy must be considered.

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