

Enzyme-linked immunosorbent assay for the detection of mycobacterial antigens in the cerebrospinal fluid in tuberculous meningitis

P. R. DONALD, ROSEMARY C. COOPER

Summary

Fifty-three cerebrospinal fluid specimens from meningitis patients were investigated by enzyme-linked immunosorbent assay for detection of mycobacterial antigens. After heating at 56°C for 1 hour to eliminate nonspecific interference, all 22 tuberculous meningitis (TBM) specimens had an optical density of greater than 0,05 (sensitivity 100%). Six out of 31 non-TBM cases gave false-positive results (specificity 81%).

S Afr Med J 1987; 71: 699-700.

Confirmation of the diagnosis of tuberculous meningitis (TBM) is dependent on culture of *Mycobacterium tuberculosis* from the cerebrospinal fluid (CSF), but the success with which this is accomplished varies considerably. Results of culture will furthermore not be available for several weeks or longer and it is vital that antituberculosis therapy be instituted as soon as possible since prognosis is intimately linked to the stage of the disease at which therapy is begun. Recently, the detection of mycobacterial antigens in the CSF of TBM patients has been described: firstly by means of an enzyme-linked immunosorbent assay (ELISA) using an anti-BCG IgG;¹ and secondly by using latex particle agglutination with a monoclonal antibody for the detection of a mycobacterial plasma membrane antigen.²

Experience with the use of anti-BCG IgG in an ELISA for the detection of mycobacterial antigen in the CSF in TBM is reported.

Material and methods

CSF from 53 patients with meningitis was evaluated. Twenty-two patients had TBM, the diagnosis being confirmed in 13 cases by culture of mycobacteria from the CSF. In the remaining 9 cases a clinical diagnosis of TBM was supported by compatible history and CSF findings together with a chest radiograph suggestive of pulmonary tuberculosis or culture of *Myco. tuberculosis* from a source other than CSF (usually gastric aspirate). The cause of meningitis in the remaining 31 cases was confirmed by culture of the relevant organisms from the CSF — *Haemophilus influenzae* in

9 cases, *Streptococcus pneumoniae* in 8 cases, *Neisseria meningitidis* in 4 cases, *Cryptococcus neoformans* in 2 cases and *Staphylococcus aureus* and a *Klebsiella* species in 1 case each, an enterovirus not further identified in 3 cases, a mumps virus in 2 cases and a Coxsackie A virus in 1 case. The CSF specimens evaluated were obtained either before the start of therapy or within 1 week in the TBM cases while the remainder were the initial diagnostic CSF specimens from which the organism was grown.

The ELISA method was that of Engvall and Perlmann³ using microtitre trays (Sterilin Products) labelled with commercially available anti-BCG (Dakopatts A/S, Copenhagen) at an optimum dilution (1:3200) in carbonate buffer pH 9,6. The reaction was labelled by peroxidase-conjugated anti-BCG dilution 1/1000 and *o*-phenylene diamine substrate. Commercial BCG (BCG, Japan) protein standards of 10 ng/ml, 5 ng/ml, 2,5 ng/ml, 1,25 ng/ml and 0,6 ng/ml gave absorbance values of 0,27, 0,19, 0,15, 0,6 and 0,04 respectively when read at 405 nm.

Results

The optical density (OD) obtained by the ELISA in the CSF specimens is illustrated in Fig. 1 before and after heating at 56°C

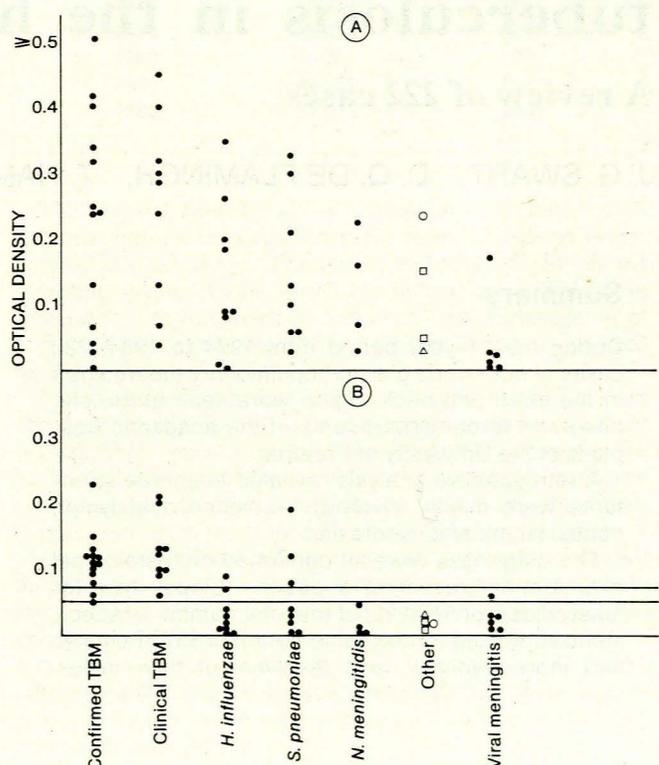


Fig. 1. Detection of mycobacterial antigen in the CSF in TBM by ELISA using anti-BCG IgG. (A) Assay with CSF untreated. (B) Assay after heating of CSF for 1 hour at 56°C (□ = *C. neoformans*; ○ = *Klebsiella* spp.; △ = *Staph. aureus*).

Departments of Paediatrics and Medical Microbiology, University of Stellenbosch and Tygerberg Hospital, Parowvallei, CP

P. R. DONALD, F.C.P. (S.A.), M.R.C.P. D.T.M. & H.
ROSEMARY C. COOPER, M.S.C.

for 1 hour in order to eliminate nonspecific interference. Before heating, a considerable overlap in values is evident. Following heating no TBM case had an OD of less than 0,05 (sensitivity 100%), however, 6 cases of non-TBM had values greater than 0,05 (specificity 81%). If 0,075 is taken as the lower limit for the detection of mycobacterial antigen, 3 cases of TBM were not detected (sensitivity 86%) while there were 4 false-positives among the non-TBM group (specificity 87%).

Discussion

Our results indicate that the relatively simple ELISA technique with readily available materials is able to detect mycobacterial antigen in the CSF in TBM. It is particularly pleasing that the clinical TBM cases also gave positive results. Four TBM specimens had an OD of less than 0,05 before heating at 56°C and it is possible that nonspecific blocking of the antigen-antibody reaction by CSF protein was responsible for this. Alternatively, specific antimycobacterial immunoglobulins, which are known to be present in the CSF in TBM,⁴ may have been responsible and were inactivated by the heating process. Given that *Myc. bovis* (BCG) has a number of antigens which it shares with other bacterial species⁵ it is perhaps not surprising that some false-positive reactions were obtained and this should be kept in mind in interpreting results.

This study was supported by the South African Medical Research Council and was undertaken by P. R. D. in partial fulfilment of the requirements for the degree M.D. at the University of Stellenbosch.

The authors thank the Medical Superintendent of Tygerberg Hospital for permission to publish, and the Department of Didactics, University of Stellenbosch, for assistance with the figure. We are indebted to Dr Agnete Ingild of Dakopatts Laboratory, Copenhagen, for the gift of peroxidase-conjugated anti-BCG.

REFERENCES

1. Sada E, Ruiz-Palacios GM, Lopez-Vidal Y, Ponce de Leon S. Detection of mycobacterial antigens in cerebrospinal fluid of patients with tuberculous meningitis by enzyme-linked immunosorbent assay. *Lancet* 1983; **ii**: 651-652.
2. Krambovitis E, McIlmurray MB, Lock PE, Hendrickse W, Holzel H. Rapid diagnosis of tuberculous meningitis by latex particle agglutination. *Lancet* 1984; **ii**: 1229-1231.
3. Engvall E, Perlman P. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen coated tubes. *J Immunol* 1972; **109**: 129-135.
4. Hernandez R, Munoz O, Guiscafere H. Sensitive enzyme immuno-assay for early diagnosis of tuberculous meningitis. *J Clin Microbiol* 1984; **20**: 533-535.
5. Minden P, McClatchy JK, Cooper R, Bardana EJ, Farr RS. Shared antigens between *Mycobacterium bovis* (BCG) and other bacterial species. *Science* 1972; **176**: 57-58.

Histologically detected extrapulmonary tuberculosis in the head and neck region

A review of 222 cases

J. G. SWART, D. Q. DE FLAMINGH, T. HAMERSMA

Summary

During the 11-year period from 1974 to 1984, 222 cases of non-meningitic extrapulmonary tuberculosis in the head and neck region were seen at the ear, nose and throat departments of the academic hospitals of the University of Pretoria.

A retrospective analysis revealed that three structures were mainly involved — the cervical lymph nodes, larynx and middle ear.

The diagnoses were all confirmed on histological examination, revealing a positive biopsy rate for tuberculosis of 5% (222) of the total number of specimens submitted for the same period (4 357). Females had more cervical node involvement than males.

Ninety per cent of all patients were black and the largest group was under 10 years old.

A high index of suspicion of tuberculosis is important in the differential diagnosis of neck swellings, hoarseness and otorrhoea.

S Afr Med J 1987; **71**: 700-702.

Extrapulmonary lesions of tuberculosis present in many and diverse ways and are defined as tuberculous lesions producing disease outside the lung. These vary from well-described clinical entities as in the genito-urinary tract and joints to nonspecific suppurative processes in soft tissue and lymph nodes.¹ However, relatively little has been reported on the occurrence and nature of non-meningitic tuberculosis in the head and neck region.

It has been established that the late forms of non-respiratory tuberculosis predominate in groups in which the risk of tuberculous infection is rapidly declining.²

Lesions in the neck, upper aerodigestive tract and ear in patients presenting to the ear, nose and throat departments of

Department of Otorhinolaryngology, University of Pretoria

J. G. SWART, M.B. CH.B., F.C.S. (S.A.)

D. Q. DE FLAMINGH, M.B. CH.B., M.MED. (ORL.)

T. HAMERSMA, B.SC., M.B. CH.B., M.MED. (PATH.)