Rapid Diagnosis of CNS Tuberculosis by a T-Cell Interferon-γ Release Assay on Cerebrospinal Fluid Mononuclear Cells

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Abstract
Central nervous system tuberculosis remains a clinical diagnostic challenge. The ex vivo Mycobacterium tuberculosis-specific enzyme-linked immunospot assay (ELISPOT) is a novel assay for the rapid detection of M. tuberculosis-specific T-lymphocytes in the peripheral blood. However, when performed on peripheral blood, this assay cannot distinguish between active tuberculosis or latent tuberculosis infection. On the assumption that M. tuberculosis-specific T-lymphocytes migrate to sites of infection, we were able to demonstrate high levels of M. tuberculosis-specific cells by ELISPOT in the cerebrospinal fluid of a patient with tuberculous meningitis and intracerebral tuberculoma four weeks before cerebrospinal fluid culture became positive for M. tuberculosis by culture.

The Case
A 38-year-old somnolent man from Sierra Leone was admitted to our hospital, with a 5-day history of increasing mental obtundation. He was a chronic HbsAg carrier, and had been treated for HCV infection with interferon-α and ribavirin until 7 months before this event. He had not left Germany for the past 7 years. On admission he had a temperature of 39 °C with a normal pulse and blood pressure. He complained of headache, was somnolent and had mild nuchal rigidity. Generalized muscle weakness was noted. Chest and abdominal examination were unremarkable. HIV serology was negative. Cerebrospinal fluid (CSF) analysis revealed mononuclear pleocytosis (590 WBC/μl [< 4/μl] with 84% lymphocytes), an elevated protein level of 5.140 mg/l [< 450], lactate of 11.2 mmol/l [2.2–2.8], and a glucose level of 2.2 mmol/l with a blood glucose of 9.6 mmol/l [quotient 0.22, normal 0.5–0.6]. CSF was sent for Gram and acid-fast stains, which were both negative, as was nucleic amplification for Mycobacterium tuberculosis (MTB)-complex DNA. Two days later these investigations were repeated with the same results. CSF was also cultured on blood and chocolate agar plates, and on liquid and solid media selective for mycobacteria. Chest X-ray was normal, while EEG showed unspecific general slowing. Cranial computed tomography revealed an occulsive hydrocephalus and multiple ring-enhancing lesions in the cerebellum and cerebral hemispheres with minor perifocal edema and without midline shift (Figure 1). Diffuse basilar meningeal enhancement was also present.

A tuberculin skin test was performed which showed an induration of 30 mm after 48 h. A MTB-specific enzyme-linked immunospot assay (ELISPOT) test (T-SPOT.TB test; Oxford Immunotec, Abingdon, UK) was performed on peripheral blood showing 216 early secretory antigenic target-6 (ESAT-6) and 250 culture filtrate protein-10 (CFP-10) interferon-γ spot forming cells (SFC) per 250,000 peripheral blood mononuclear cells (PBMCs). More than 5 SFC/250,000 PBMCs is regarded as a positive result by the manufacturer. In addition, T-SPOT.TB test was also performed on mononuclear cells from the CSF demonstrating a high frequency of MTB-specific lymphocytes of 223 ESAT-6 and 296 CFP-10 specific SFC per 250,000 mononuclear cells in the CSF, respectively (Figure 2). Mononuclear cells from the CSF were obtained by centrifugation of the CSF fluid and resuspension of the cells in RPMI 1640 culture medium. Positive ELISPOT results were confirmed at another laboratory.

On the basis of the clinical presentation and laboratory results, the patient was treated with quadruple antituberculous chemotherapy (isoniazid [INH], rifampin [RIF], ethambutol [EMB] and pyrazinamide [PZA]) for presumptive CNS tuberculosis and triple antibiotic therapy (ceftriaxone, vancomycin, ampicillin) for possible bacterial meningitis. In addition, dexmethasone 12 mg/day was started. One week later the patient developed severe liver function abnormalities leading to discontinuation of INH, RIF and PZA. Instead streptomycin, moxifloxacin and linezolid were given together with EMB. After
the liver function had returned to preadmission levels treatment with RIF and PZA was reintroduced without reoccurrence of a hepatitis and treatment with streptomycin and linezolid was discontinued.

Four weeks after initiation of therapy CSF cultures grew MTB fully sensitive to isoniazid, ethambutol, rifampin, pyrazinamide and streptomycin.

The patient improved slowly and could be discharged to a rehabilitation hospital 12 weeks after admission with an antituberculous medication including RIF, PZA, moxifloxacin and EMB.

**Discussion**

The diagnosis of tuberculous meningitis remains a severe problem in clinical practice [1]. This case illustrates the potential advantages of measuring local mycobacterial-specific immune responses in the CSF for the rapid diagnosis of CNS tuberculosis in clinical practice, particularly when classical tests such microscopy for acid-fast bacilli, or nucleic acid amplification technologies for MTB-specific nucleic acids are negative and microbiological culture results are not yet available [2].

In developed countries tuberculous meningitis is rare (less than 5% of all patients with bacterial meningoencephalitis). The disease frequently starts with nonspecific symptoms such as fatigue, a general feeling of sickness, slight elevation of body temperature, headache, nausea, behavioral changes including restlessness. When nuchal rigidity develops, approximately 80% of patients suffer from headache and fever, but approximately 20% of patients never develop fever. About 20–40% of patients have cranial nerve involvement (most frequently Nn. II, III, VI, VII and VIII) [1, 3]. Some patients present with spinal radicular lesions, hemi- or para- or even tetraparesis as initial symptoms. An elevation of intracranial pressure and subsequent development of occlusive hydrocephalus caused by an impaired CSF circulation is frequent and a risk factor for poor outcome [3]. Lumbar puncture typically reveals a moderate CSF pleocytosis of several hundred leukocytes/μl (lymphocytes, monocytes and granulocytes) a strong elevation of CSF protein (> 1,000 mg/l; normal below 450 mg/l) and lactate (> 3.5 mmol/l, normal < 2.2–2.8 mmol/l), and a decreased CSF-to-blood glucose ratio (< 0.4; normal 0.5–0.6) [4]. Similar CSF abnormalities can be found in CNS cryptococcosis, listeriosis, and meningitis neoplastica, the most frequent disease in developed countries mimicking tuberculous meningitis [5]. Here, analysis of CSF cytology by an experienced pathologist is necessary to document tumor cells [6].

The outcome of untreated tuberculous meningitis is almost inevitably fatal. Because of the low sensitivity of

![Figure 1. Cranial computed tomography scan with intravenous contrast of the brain obtained on day 10 after admission. Multiple up to 1 cm sized ring-enhancing lesions with perifocal edema are found in the cerebellum and cerebral hemispheres consistent with tuberculomas. An enlargement of the ventricles indicating occlusive hydrocephalus is visible. Decreasing levels of consciousness led to an external ventriculostomy on day 30 which was replaced by a ventricular-peritoneal shunt before discharge.](image1)

![Figure 2. MTB specific ELISPOT with ESAT-6 and CFP-10 antigen on mononuclear cells from the cerebrospinal fluid. For the MTB-specific ELISPOT (T-SPOT.TB, Oxfordimmunotec, Abingdon, UK), 2.5 x 10^5 mononuclear cells are isolated from CSF in microtiter plate wells in the presence of ESAT-6 and CFP-10 or PHA (positive control) or in the absence of antigen (negative control). Antigen-reactive T-cells in the wells release interferon-γ upon antigen contact. Subsequently interferon-γ is bound to an anti-interferon-γ-specific antibody at the bottom of the wells. After counterstaining with a second soluble anti-interferon-γ-specific monoclonal antibody and a color reaction, spots are visible on the bottom of the plates at locations where single cells have produced interferon-γ following antigen contact. Test results are available within 24 h. On PBMCs the recommended cutoff for a positive response is 5 spot forming cells (sfc)/250,000 mononuclear cells. There is no accepted cutoff value for extra-sanguinous fluids currently.](image2)