Epstein-Barr virus associated with primary CNS lymphoma and disseminated BCG infection in a child with AIDS

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Summary

Background: AIDS patients are at increased risk of developing concurrent infections with viral, parasitic, fungal or mycobacterial organisms. They can present constitutional symptoms of fever and weight loss, either due to infections or an underlying lymphoma which may coexist.

Case report: A child with HIV-AIDS and mild encephalopathy is reported, who during the course of a confirmed disseminated mycobacterial disease developed neurological impairment. Post-mortem examination revealed disseminated BCG infection and Epstein-Barr associated primary CNS lymphoma. Epstein-Barr virus (EBV) presence was assessed by LMP-1 protein labelling by immunohistochemistry and in situ hybridisation (ISH) for Epstein-Barr virus-encoded RNAs (EBERs) in formalin-fixed and paraffin-embedded sections.

Conclusions: BCG vaccination among HIV-1 infected children leads to the risk of disseminated BCG infection. BCG immunization programmes should be reconsidered for children at risk of HIV infection, because the risk of delayed complications is independent of the immunological status at the time of the vaccination.
Introduction

Human immunodeficiency virus infection produces a broad spectrum of diseases in both children and adults. AIDS patients are at increased risk of developing opportunistic infections. Patients may present symptoms of fever and weight loss, either due to infections or a coexisting lymphoma. In children, opportunistic, reactivated latent infection and CNS lymphoma have been infrequent according to neuroradiological and autopsy examination. The most frequent CNS complication is progressive encephalopathy attributable to primary HIV infection of the brain.1

Primary CNS lymphoma represents a frequent complication of HIV infection in adults, occurring in as many as 6—10% of patients with AIDS, however, only isolated cases of primary CNS lymphoma occurring in HIV-infected children have been reported,2—5 and a striking association with Epstein-Barr virus (EBV) infection has been demonstrated.5—7

Disseminated infection by bacillus Calmette-Guérin (BCG) is a rare but severe complication of BCG vaccination in immunodeficient patients. There is an increasing number of reports on this topic. Local or disseminated BCG disease has historically been a disease of infants, but cases in adults and older children with AIDS have been reported.5—9

A case is reported here of a child with AIDS with a mild HIV encephalopathy, who during the course of confirmed disseminated mycobacterial disease developed neurological impairment. Post-mortem examination revealed disseminated BCG infection and Epstein-Barr virus associated primary CNS lymphoma.

Material and methods

Immunohistochemistry (IHC)

The detection of latent membrane protein-1 (LMP-1) of EBV was performed using a pool of four monoclonal antibodies (MoAbs) CS1-4 (Dako Corporation, Carpinteria, CA, USA). Immunohistochemical detection on formalin fixed paraffin embedded tissues employed the streptavidin-biotin complex with horseradish peroxidase technique according to the manufacturer’s instructions (Dako Corporation, CA, USA).

In situ hybridization (ISH)

Fluorescein isothiocyanate (FITC)-conjugated Epstein-Barr encoded RNA (EBERs) oligonucleotides were used as probes for the hybridization procedure on paraffin sections. EBERs ISH was performed as previously described by Preciado et al.10 Detection of the hybridized sites was achieved using a monoclonal antibody anti-FITC labelled with alkaline phosphatase (Novocastra, Newcastle-upon-Tyne, UK), performed according to the manufacturer’s instructions.

Polymerase chain reaction assay for Mycobacterium tuberculosis

Preparation of DNA: Samples submitted were sputum, CSF and blood. Sputum was decontaminated and digested by the NALC-NaOH method.11 CSF was concentrated by centrifugation. Both samples were then treated with proteinase K. Peripheral blood mononuclear cells were separated and DNA was obtained.

PCR: Amplification of DNA was designed as nested-PCR using the oligonucleotides described by Eisenach and Nolte as primers IS-1 (884—865), IS-2 (568—588) and IS-3 (762—781).12,13 Primer IS-4 (910—891) was personally selected from the complete sequence published by Eisenach. The amplified region (123 bp) belongs to the IS6110 repeated sequence which is specific for Mycobacterium tuberculosis complex (M. tuberculosis, M. bovis, M. africanum and M. microti).11,12 Both PCRs were performed in a final volume of 50 µl containing 1.5 mM MgCl2; 200 µM (each) dNTPs; 0.5 µM (each) primers; 1.25 U of Taq DNA polymerase; 1 x PCR buffer provided with the enzyme, and 5 µl of lysis supernatant. The reaction was made in a touchdown system from 70 °C to 68 °C for the first round (IS-2-IS-4) and 74 °C to 68 °C for the second (IS-1-IS-3). Inhibition was also evaluated.

The following biochemical characteristics were used to identify M. bovis-BCG: negative niacin test;
absence of nitrate reductase; susceptibility to thiophene-2-carboxylic acid; and resistance to pyrazinamide and cycloserine.

Case report

A 30-month-old girl with AIDS was admitted to the Hospital de Niños ‘Ricardo Gutiérrez’ in Buenos Aires due to diarrhea and dehydration. Perinatally-acquired HIV infection was diagnosed at three months of age. Her clinical history included *Pneumocystis carinii* pneumonia, recurrent oral candidiasis, chronic diarrhea and bacterial pneumonia. By 12 months of age she had neurodevelopmental delay, hyperreflexia and a CT scan showed cerebral atrophy. She had received BCG vaccine in her first month of life. She was treated with different antiretroviral protocols because of clinical, immunological and virological progression. A month prior to admission she had been studied because fever and a chest X-ray showed persistent atelectasis of the right upper lobe and mild diffuse interstitial pulmonary infiltrates without respiratory impairment. Twenty units of PPD administered intradermally was negative. *Mycobacterium tuberculosis* complex DNA was detected by PCR in the blood, and therapy was started with isoniazid, ethambutol, pyrazinamide and amikacin.

She had no known exposure to adults with tuberculosis. Multiple studies failed to demonstrate TB in her household contacts.

On admission she had diarrhea, fever and severe dehydration, oral thrush, mild hepatosplenomegaly and weight loss; her growth and development were delayed, she was alert and her mental status was not affected. She was on PCP prophylaxis with trimethoprim/sulphamethoxazole and antiretroviral therapy affected. She was on PCP prophylaxis with trimethoprim/sulphamethoxazole and amikacin.

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capsule and in the anterior corpus callosum (Figure 2).

Differential diagnoses included disseminated tuberculosis, primary HIV encephalopathy, toxoplasmosis, CNS lymphoma or CNS fungal infection. A cerebral biopsy was planned but could not be done because the patient’s clinical condition worsened. She died on the 71st hospital day.

Figure 1  Initial CT scan showing a small rounded hyperdense lesion surrounded by a low density zone (edema) that causes compression of the left frontal horn and a high density lesion in the basal ganglia. Mild diffuse cortical atrophy is seen.

Figure 2  Last CT scan showing diffuse cortical atrophy with enlarged ventricles and decreased lesion size in basal ganglia. Enhanced diffuse zone in both occipital lobes. Hypodense lesion in the right posterior internal capsule and left subinsular region, without mass effect.
Post-mortem examination revealed a diffuse CNS lesion that involved brain parenchyma and bilateral ventricles, with multiple cystic lesions in the temporal lobules and a nodular lesion in the basal ganglia area. Histological examination disclosed atypical lymphoid cells with a predominant perivascular arrangement that involved cerebellum, brain stem and spinal cord (Figure 3). Large foci of ischaemic necrosis with calcifications and large intraparenchymatous and intraventricular haemorrhage were found. No microorganisms were detected with PAS and Ziehl-Nielsen staining. Immunohistochemically, expressed B-cell antigen (CD20) and CD3, CD15, CD30, CD68 were negative. These results showed the diagnosis of this tumor to be a primary CNS diffuse large B cell lymphoma (Figure 4).

The enlarged spleen showed small multiple nodules which histologically were granulomatous

Figure 3  Photomicrograph of brain specimen showing angiocentric distribution of atypical small lymphocytes with angulated or cleaved nuclei (hematoxylin and eosin 250×).

Figure 4  Immunohistochemical studies revealing a predominant B-cell population (CD 20) (400×).
lesions without necrosis, with abundant intracellular acid-fast bacilli. Similar lesions were found in mesenteric lymph nodes. Examination of the lung revealed interstitial pneumonitis. BCG was cultured from the tibial lesion.

**EBV results**

EBERs in situ hybridization (ISH) showed intense nuclear labelling tending to spare the nucleolus. LMP-1 positive staining was detected in tumor cells localized to the cell membrane and cytoplasm (Figure 5).

**Discussion**

This case showed a co-infection with three aggressive pathogens in one patient. The primary HIV infection, which progressed to AIDS, and a secondary infection of a disseminated BCG infection with the occurrence of an EBV-associated CNS lymphoma.

This case emphasizes the need for invasive diagnostic methods to disclose concomitant etiologies. In this particular case, a brain biopsy could not be carried out because of the deep location of the lesion and the critical clinical status of the patient. Only post-mortem examination revealed the final diagnosis.

Disseminated BCG infection is a long-known entity with numerous reports published prior to the AIDS era, especially from European countries. A French national retrospective study reported 0.59 cases of disseminated BCG infection/1 million vaccinated healthy children.\(^\text{14}\)

Lotte reported 2 cases/1 million with a mortality rate of 80%, in a retrospective study from six European countries.\(^\text{15}\) Cellular immunodeficiency was identified as the chief risk factor for fatal outcome.\(^\text{15,16}\) Tuberculous osteitis is one of the most frequent complications of the BCG vaccination.\(^\text{17,18}\)

In severe immunodeficiency, there is a lack of granuloma formation and unimpeded proliferation of acid-fast bacilli and variable or absent necrosis.\(^\text{19,20}\) Usually these patients have specific unresponsiveness to tuberculin tests.\(^\text{21}\) The case here showed both histopathological findings, as there was necrosis in the osteal lesion, but it was absent in the spleen.

PCR led to the diagnosis of disseminated *Mycobacterium tuberculosis* complex infection one month before the beginning of bone symptoms in the reported patient. The sensitivity, specificity and positive predictive value of PCR when applied to pediatric samples for diagnosing tuberculosis are 78.4%, 96% and 97% respectively,\(^\text{22}\) and no cross reaction with mycobacteria other than *M. tuberculosis* complex has been observed.\(^\text{23,24}\)

In spite of some studies that report a small risk of complications following BCG vaccination among HIV-1 infected children and encouraged the benefits of BCG vaccination,\(^\text{25,26}\) there are several reports of severe, including fatal, disseminated BCG infection occurring in immunodeficient patients.\(^\text{9,27}\)
Therefore, BCG immunization programmes should be reconsidered for children at risk of HIV infection. Immunocompromized patients with late-stage AIDS are at great risk, with an overall 71% mortality reported.29 Both this case, and similar cases reported, emphasize the hazards of BCG vaccination in HIV-infected infants.

This is the first case of primary CNS lymphoma seen at the Hospital de Niños Ricardo Gutiérrez in Buenos Aires, Argentina, among 351 HIV-infected children followed up prospectively.29 This represents a frequency lower than 1%, which is similar to the 1996 CDC report of 30 children with CNS lymphoma, among 7629 children with AIDS, which represented a frequency of 0.4%. In addition, the AIDS-Cancer Match Registry (July 2000) reported a frequency of 0.5%.30,31 Only a few cases of primary central nervous system NHL (non-Hodgkin’s lymphoma) occurring in children with HIV infection have been reported so far.2–5 Other CNS NHL associated with EBV have not yet been reported in Argentina.

The Epstein-Barr virus may be responsible for the lymphoproliferative disorders described in HIV infected children.32,33 When EBV infects B-lymphocytes, it induces cell proliferation. Such lymphoproliferative action may enable oncogenesis by increasing the probability of genetic alterations (e.g., chromosomal translocations such as those seen in Burkitt lymphoma) and/or by expanding an already malignant clone.7 Expression of LMP-1, an oncogenic protein, by tumor cells supports the involvement of EBV in disease pathogenesis. It is well known that primary CNS lymphoma occurs with increased frequency in adult patients with AIDS and they are nearly always EBV positive.34

Like the case reported here, most patients died very soon after the time of onset of symptoms related to the lymphoma, suggesting the need of both rapid diagnostic procedures and treatment. Although the clinical outcome with current treatments remains rather discouraging, there have been some recent reports describing a more positive prognosis.35,36

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