Neonatal Cryptococcosis: Beware of False-positive Results

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Although cryptococcal meningitis is uncommon in children and rare in neonates, it does occur. We
highlight circumstances in which the diagnosis should be considered and methods required to confirm
the diagnosis in young patients.

A one month old female infant was brought to Rahima
Moosa Mother and Child Hospital, Johannesburg, South
Africa with non-specific symptoms of crying and irritabil-
ity by a social worker before being taken to a place of
safety. Her mother had been admitted to the local adult
hospital, but her diagnosis was not known at that time.
Further history was unavailable, however, it was known
that the baby was HIV-exposed and was living in poor
social conditions. According to the social worker, she
had been crying inconsolably, feeding poorly and had
vomited several times. On examination, she was symme-
trically growth restricted: weight, height and head circum-
ference were all below 2 standard deviations under the
mean for term infants. There were no dysmorphic fea-
tures. She was noted to have oral candidiasis, wasting,
sparse hair, mild napkin dermatitis and was pyrexial with
a temperature of 38.5°C. The cardio-respiratory exam-
ination was normal. On neurological examination, she was
irritable with hypertonicity, but no neck stiffness was
present. Fontanelles were open but not bulging.

Laboratory investigations revealed a white cell
count of 14.5x10^9/l, haemoglobin level of 10g/dl and
thrombocytosis of 524x10^9/l. Blood and urine cultures
were negative. Chest x-ray showed a bilateral patchy
nodular infiltrate with air bronchograms. Hilar lymph-
adenopathy was difficult to ascertain owing to the
infiltrate. The tuberculin skin test (TST) was non-
reactive (0mm). HIV-DNA PCR was positive with a
CD4 count of 1013x10^6/l (18%). A lumbar puncture
was performed and cerebrospinal fluid (CSF) analysis
revealed 20 polymorphonuclear cells, 10 lymphocytes
and >10000 erythrocytes/mm^3 (this was considered
consistent with a traumatic spinal tap); protein 0.58g/l,
and glucose 3.0mmol/l. A Gram stain did not reveal
the presence of any bacteria. Considering that her
HIV-infected mother had been admitted with an
unknown diagnosis and that she had clinical features
consistent with meningitis, an India ink stain and
cryptococcal latex antigen test (CLAT) were requested.
The CSF India ink was negative but CSF CLAT was
positive with a titre of 16.

Treatment with intravenous amphotericin B was
initiated and a blood specimen for CLAT was sent. This
CLAT was also positive though no titres were reported.
CLATs were repeated on these initial CSF and blood
specimens and the results confirmed. A second lumbar
puncture repeated 48 hours after initiation of antifungal
therapy, had 0 polymorphonuclear cells, 11 lympho-
cytes and 14 erythrocytes/mm^3, protein 0.26g/l, glucose
3.0mmol/l and was CLAT negative; Blood cytomegalov-
irus (CMV)-PCR was positive with a CMV viral load of
17 000 copies/ml. It was suspected that the initial CSF
result was as a result of antigen present in the blood
mixed with CSF and that the child did not in fact have
cryptococcal meningitis. All the available blood and
CSF specimens from the mother and baby, including
the initial positive CSF specimen, were sent to the
National Health Laboratory Services, Mycology
Reference Unit for lateral flow assay (LFA) and enzyme
immunoassay (EIA) which confirmed the absence of
cryptococcal antigen in all specimens examined.
Cultures on both CSF specimens were negative for
cryptococcus and other pathogens. Amphotericin B was discontinued after 7 days of therapy. The infant was treated for bacterial meningitis with intravenous ceftriaxone for 8 days, and highly active antiretroviral therapy (HAART) was then initiated. The fever resolved and her weight and clinical condition improved. She was discharged 17 days after admission. Mycobacterial culture of blood and CSF were negative.

It subsequently emerged that the child’s mother had pancytopenia and a pleural effusion and was being investigated for mycobacterial disease. She was not being treated for cryptococcosis and her blood cryptococcal latex antigen test was negative.

**DISCUSSION**

Cryptococcosis is a systemic mycosis which is the commonest cause of meningitis in the adult HIV population in South Africa [1]. Available data suggest it is uncommon in children and rare in neonates – Gonzalez et al. reported an incidence of 0.85% among 473 children over 8 years. All patients were severely immunocompromised and older than 10 years of age [2]. A Malawian case series published in 1997 described the first three African paediatric cases in patients aged 6 weeks to 9 years [3] and an 2002 Indian report is believed to be the first described neonatal case of cryptococcal meningitis [4]. The route of transmission to neonates remains uncertain. Since *Cryptococcus neoformans* is ubiquitous in the environment, repeated exposure may be one mode of transmission [5]. Vertical transmission has only been described twice before [6, 7], where the mothers presented with pneumonia and/or central nervous system manifestations of cryptococcosis and the neonates subsequently grew *C. neoformans* on blood culture. A Brazilian case report described a case of transmission from mother to child by probable peripartum haematogenous dissemination [7]. Neonatal cases occur predominantly in premature infants.

A retrospective cohort study between 1985 and 1996, investigated 30 children (median age 9.8 years) and found increasing incidence of cryptococcosis with age and worsening immune function [5]. Thus, clinical suspicion should be raised in severely immunocompromised older children presenting with fever and headaches.

Most laboratories rely on India ink staining, cryptococcal latex agglutination and organism culture for diagnosing cryptococcosis, with culture considered the gold standard. Commercially available latex-agglutination tests have a sensitivity and specificity of

<table>
<thead>
<tr>
<th>Cause</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune</td>
<td>Rheumatoid factor in patients with rheumatoid arthritis</td>
<td>Immunoglobulin M and anti-immunoglobulin G antibodies may be present in serum, and rarely in cerebrospinal fluid (CSF)</td>
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<td>Infective</td>
<td><em>Trichosporon beigelii</em></td>
<td>These organisms produce polysaccharides in their capsules which cross react with anti-cryptococcal antibodies</td>
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<td></td>
<td><em>Capnocytophaga canimorsus</em></td>
<td>Reported only once, the cause of this cross-reaction is not known</td>
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<td></td>
<td><em>Stomatococcus mucilaginosis</em></td>
<td>Up to 2% of human immunodeficiency virus–infected adults may have a positive latex agglutination test in the absence of disease</td>
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<tr>
<td>Metabolic</td>
<td>Serum Fe^{3+} &gt;200mg/dL</td>
<td>Very low titres</td>
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<tr>
<td>Malignancy</td>
<td>Astrocytoma</td>
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<td>Ewing sarcoma</td>
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<td>Lung carcinoma</td>
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<td></td>
<td>Malignant melanoma</td>
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<tr>
<td>Substance</td>
<td>Low molecular weight hydroxyethyl starch</td>
<td>Polysaccharide cross reaction</td>
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<td>for intravascular volume replacement during fluid resuscitation</td>
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<tr>
<td>Specimen handling</td>
<td>Contamination during extraction of specimen sample for testing</td>
<td>When immersing platinum wire loops in CSF, care should be taken not to contaminate the sample with syneresis fluid (surface condensation) from chocolate agar plates, which even in small amounts can cause false-positive reactions. Therefore the CSF latex antigen test should be performed before culturing or from separate tubes</td>
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<tr>
<td></td>
<td>• during pipetting</td>
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<td></td>
<td>• using platinum loop</td>
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<td></td>
<td>Soaps and disinfectants used in slidewash</td>
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<td></td>
<td>Talc powder contamination from latex gloves</td>
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Table. Causes of False-positive Cryptococcal Latex Agglutination Tests [8]
93-100% and 93-98% respectively for CSF specimens [9]. False-positive test results may occur (see Table), but are uncommon [8]. It is recommended that specimens should be pre-treated with proteolytic enzymes (pronase) and reduced by use of 2-β-mercaptoethanol or dithiothreitol to avoid cross-reactions resulting from the causes described in the Table. The NHLS laboratory which performed the CLATs on our specimens routinely pre-treat all serum and blood-stained CSF specimens with pronase.

Soaps and disinfectants used in glass slide washing and contaminated pipettes, used for specimen transfer may cause false-positive reactions, however, our laboratory uses manufacturer provided test-cards instead of glass slides, and sterile pipettes. Apart from HIV infection, none of the autoimmune, infective, malignant or substance cross reactions were implicated in our patient and we thus postulate that this false-positive reaction was probably related to HIV infection.

Cryptococcal LFA is a highly sensitive and specific diagnostic test and given the ease of use, minimal laboratory requirements, potential low cost and temperature stability, it shows great promise as a Point of Care test for the diagnosis of cryptococcosis [10]. When infantile cryptococcosis is suspected, an effort should be made to confirm it by evaluating the mother’s health, repeating the test result in CSF and/or blood and by sending the specimens for additional confirmatory testing. Conceivably, false-positive CLATs may detect exposure to transplacentally acquired maternal antigen in the absence of infection. Contamination of clinical specimens may also result in false-positive test reactions. Once the diagnosis is confirmed or there is strong clinical suspicion which warrants therapy, there are available guidelines for treatment of older children and adults [11] but limited guidance for treating infants and young children. A case report from China of a 4 month old premature baby with cryptococcus detected on CSF antigen and culture, describes successful treatment with amphotericin B plus flucytosine for two weeks, followed by high dose fluconazole for 8 weeks and lower dose maintenance fluconazole thereafter [12]. We opted to initially treat with amphotericin B as per national adult guidelines [11]. Evidence suggests better outcomes with initial amphotericin B (with or without 5-flucytosine) compared to fluconazole therapy alone [113].

In conclusion, although cryptococcosis is common in the adult HIV population, it is uncommon in children and extremely rare in neonates. The reason that it may be so rare is that serological studies suggest that exposure usually occurs after the first two years of life [14]. Another reason may be the difference in immune response. However the diagnosis may be considered in premature infants, infants of mothers with confirmed cryptococcosis, and more severely immunocompromised older children and adolescents. While usually highly specific, positive cryptococcal latex antigen tests in the absence of confirmatory microscopic or culture results should be treated with caution as false-positive results do occur. Cryptococcal LFA could be considered before administering extended courses of treatment.

Acknowledgments

(1) Ranmini Kularatne, Microbiologist, Helen Joseph Hospital, NHLS, for information regarding causes of false positive cryptococcal latex antigen tests and method of pre-treating specimens at NHLS. (2) Mycology Reference Unit, National Institute for Communicable Diseases (NICD), Johannesburg, for performing the Lateral Flow Assay.

References


