

## CASE REPORT

# MICROSCOPY 'AIDS' IN DIAGNOSING A FEBRILE INFANT

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A 5-month-old South African girl presented to a casualty department with a short history of fever. General examination did not reveal organomegaly or neck stiffness. In keeping with local guidelines, malaria was excluded on antigen testing and microscopy (thick and thin smear with Giemsa stain).<sup>1</sup> *Rickettsia typhi*, *R. conorii* and *Coxiella burnetii* were also excluded on the basis of serological testing.

The full blood count revealed pancytopenia with a haemoglobin (Hb) level of 7.9 g/dl (normal 10 - 15 g/dl), a white cell count (WCC) of  $1.17 \times 10^9 / l$  (normal  $5.50 - 18.00 \times 10^9 / l$ ) with an absolute lymphocyte count of  $0.80 \times 10^9 / l$ , and a platelet count of  $47 \times 10^9 / l$  (normal  $140 - 350 \times 10^9 / l$ ). Biochemical values were suggestive of renal dysfunction, with a serum urea level of 9.6 mmol/l (normal 1.4 - 5.0 mmol/l) and a creatinine level of 180  $\mu\text{mol/l}$  (normal 14 - 34  $\mu\text{mol/l}$ ), as well as a markedly elevated C-reactive protein level of 243.1 mg/l (normal 0.1 - 7.5 mg/l).

On investigation of the patient's immune status, it was established that she was HIV-1-positive as confirmed by HIV-1 DNA Amplicor assay version 1.5 (Roche Diagnostics, Mannheim, Germany), with an HIV-1 viral load of 2 738 930 RNA copies/ml by Abbott m2000 Real Time HIV-1 Assay (Abbott Diagnostics, Johannesburg) and a CD4+ lymphocyte percentage of 28%.

Initial standard paediatric blood cultures did not yield any growth after 7 days of incubation. A follow-up full blood count, performed 2 days after the initial investigations and assayed on the ADVIA 2120 (Bayer HealthCare, Diagnostic Division, Isando, Gauteng), differed markedly from the previous results, showing a WCC of  $13.83 \times 10^9 / l$  with an absolute lymphocyte count of  $10.99 \times 10^9 / l$ . On microscopic examination of the peripheral blood smear (Fig. 1, a) these counts could not be verified and the leucocytes appeared markedly reduced. In addition, fungal elements could be demonstrated (Fig. 1, a and b).

Subsequent blood cultures, performed in paediatric bacterial blood culture bottles, confirmed the aetiological agent to be *Candida albicans*. Fungal morphology clearly showed the presence of oval yeast forms, slightly smaller than a lymphocyte nucleus (Fig. 1, a), with blastoconidia and pseudohyphae (Fig. 1, b). The fungus was identified by demonstrating formation of a germ tube on 4-hour incubation in horse serum.<sup>2</sup> Subsequent antifungal susceptibility, performed on the Vitek 2 system (BioMerieux, Randburg, Gauteng), demonstrated sensitivity to all antifungal agents tested (amphotericin B, fluconazole and voriconazole).

### DISCUSSION

HIV-1 infection has been established as a risk factor for fungaemia in both children<sup>3</sup> and adults.<sup>4-5</sup> The routine use of fungal blood cultures

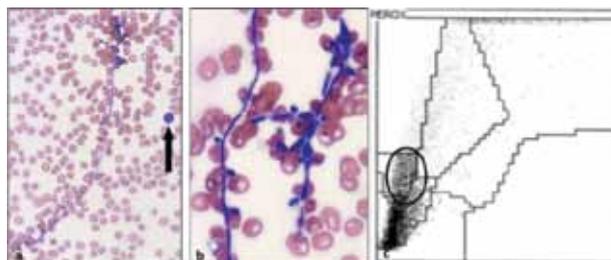


Fig. 1. Peripheral blood smear from a 5-year-old HIV-1-positive girl, showing marked fungaemia: a – size of fungal element in relation to lymphocyte (arrow); b – closer view of fungal elements, showing oval yeast forms with blastoconidia and pseudohyphae; c – peroxidase channel on ADVIA 2120 analyser. The encircled area depicts the region where lymphocytes are typically noted based on both size and peroxidase negativity. In this instance, these events are produced by yeast cells rather than lymphocytes.

remains controversial, being advocated by some authors<sup>6</sup> and discouraged by others.<sup>7</sup>

Microscopic examination of the peripheral blood smear revealed a significant leucopenia, despite contradicting automated counts. WCCs as well as differential leucocyte counts have been described to be influenced by high fungal loads of *Candida* spp.<sup>8</sup> The ADVIA 2120 uses both a lobularity and a peroxidase channel for leucocyte identification and quantification.<sup>8-9</sup> In this case a comparison error had been flagged, indicating the probability of falsely elevated counts. This error, however, does not reflect on the laboratory report issued to the clinician, and is an analytical issue that needs to be addressed by the technologist and pathologist in the laboratory setting. Owing to their small size as well as their peroxidase-negative properties, the yeast cells had been identified as lymphocytes by the automated analyser (Fig. 1, c).

Considering the increased prevalence of fungal infections among HIV-1-positive patients,<sup>3-5</sup> full blood count evaluation is best accompanied by morphological evaluation to detect any discrepancies between automated counts and actual observation.<sup>8-9</sup> In most laboratory settings, morphology is evaluated once the clinician requests a differential count. Clinicians should further request differential counts once any full blood count parameters show significant abnormalities, or when there is a clinical suspicion of disease affecting the haematological system, including bleeding tendencies, infection, hepatomegaly, splenomegaly, lymphadenopathy, etc.<sup>10</sup>

In this clinical case, the rapid change in results over a 2-day period should have prompted the clinician to suspect an analytical issue with the samples in question.

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