

Disseminated Cryptococcal Disease of the Bone Marrow, Cerebrospinal Fluid and Blood in an ART-Naïve Patient with Advanced HIV: A Case Report

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Abstract

Background: Cryptococcosis affects 10-30% of patients with acquired immunodeficiency syndrome (AIDS) resulting in significant morbidity and mortality. The primary sites of cryptococcal disease are the lung and central nervous system with bone marrow involvement being rare.

Case presentation: This case report describes a human immunodeficiency virus (HIV) positive, anti-retroviral therapy (ART) naïve female with pancytopenia who demonstrated no significant symptoms nor clinical signs as well as a negative serum cryptococcal antigen (CrAg) test. A bone marrow biopsy was then performed which resulted in a diagnosis of disseminated cryptococcal disease of the bone marrow. Blood and cerebrospinal fluid (CSF) fungal cultures confirmed the presence of *Cryptococcus neoformans*. The patient was then successfully treated with antifungal agents- amphotericin B and fluconazole.

Conclusion: Bone marrow examination may therefore be a useful method in early diagnosis of disseminated cryptococcosis as a cause of cytopenias in immunocompromised patients, thus enabling early appropriate treatment and improved outcomes.

Keywords: *Cryptococcus*; Bone marrow; Human immunodeficiency virus; Acquired immunodeficiency syndrome

Abbreviations HIV: Human Immunodeficiency Virus; AIDS: Acquired Immunodeficiency Syndrome; CrAg: Cryptococcal Antigen; ART: Anti-Retroviral Therapy; CSF: Cerebrospinal Fluid; MGG: May-Grünwald Giemsa; H&E: Haematoxylin and Eosin; PAS: Periodic Acid-Schiff; GMS: Grocott's Methenamine Silver

Background

Fungal infections are among the most common opportunistic infections in human immunodeficiency virus (HIV) positive patients [1]. Cryptococcal disease affects 10-30% of patients with acquired immunodeficiency syndrome (AIDS) and causes significant morbidity and mortality [2]. The primary sites of cryptococcal disease are the lung and central nervous system with bone marrow involvement being rare. [3,4] This is a case report of disseminated cryptococcal disease of the bone marrow in a HIV positive female who presented with pancytopenia.

Case Presentation

A 25 year old female patient was referred from a local clinic and admitted to Groote Schuur Hospital, medical department, on 08/11/2016 for evaluation of a one-month history of pancytopenia.

The patient demonstrated no significant symptoms on presentation with normal respiratory and neurological examinations and no clinical source of infection. She was known to be HIV positive with a CD4 count of 44 cells/ μ L and a viral load of 73,596 RNA copies/mL. A negative serum cryptococcal antigen (CrAg) test was detected on reflex screening. She was anti-retroviral therapy (ART) naïve. The patient's blood test parameters are listed below in Table 1.

The peripheral blood smear showed normocytic normochromic red cells with rouleaux formation, vacuolated neutrophils and reactive lymphocytes. Electrolytes, renal function and liver function tests were all within normal limits. Blood cultures (bacterial, mycobacterial/fungal) were done. A lumbar puncture was also performed with cerebrospinal fluid (CSF) submitted for cultures. However no CSF CrAg test was requested.

The next day, on 09/11/2016, the patient was referred to haematopathology for a bone marrow biopsy to investigate the cause of the pancytopenia. Bilateral bone marrow aspirates and trephines were obtained using an 11 gauge bone marrow needle. Aspirate slides were stained with May-Grünwald Giemsa (MGG) and the trephine with haematoxylin and eosin (H&E).

The aspirate demonstrated hypocellular particles surrounded by proteinaceous haloes with gelatinous transformation. Haemopoiesis revealed trilineage hypocellularity with mild dysplasia of all three cell lines. The myeloid:erythroid (M:E) ratio was 9:1. The trephine was hypocellular, estimated at 55%, with trilineage hypocellularity. The architecture was disrupted by multiple granulomas of various sizes,

with the largest measuring approximately three quarters of a 50 × high power field. These granulomas comprised of histiocytes and multinucleated giant cells, with centrally aggregated nuclei (Figure 1).

	Blood Test	Result	Reference Range
Full blood count	Total white cell count ($\times 10^9/L$)	3.02 ↓	3.90-12.60
	Haemoglobin (g/dL)	7.7 ↓	12.0-15.0
	Mean corpuscular volume (fL)	88.2	78.9-98.5
	Mean corpuscular haemoglobin (pg)	31.3	26.1-33.5
	Platelets ($\times 10^9/L$)	52 ↓	186-454
Differential count	Neutrophils ($\times 10^9/L$)	2.44	1.60-8.30
	Lymphocytes ($\times 10^9/L$)	0.38 ↓	1.40-4.50
	Monocytes ($\times 10^9/L$)	0.13 ↓	0.20-0.80
Reticulocyte count	Absolute reticulocyte count ($\times 10^{12}/L$)	0.017 ↓	0.050-0.100
	Corrected reticulocyte count (%)	0.33 ↓	0.50-2.00
	Reticulocyte production index	0.2 ↓	1-2
Iron studies	Serum iron ($\mu\text{mol}/L$)	2.6 ↓	9.0-30.4
	Transferrin (g/L)	0.99 ↓	2.00-3.60
	Transferrin saturation (%)	10 ↓	15-50
	Ferritin ($\mu\text{g}/L$)	491 ↑	13-150

Table 1: Blood test parameters of patient.

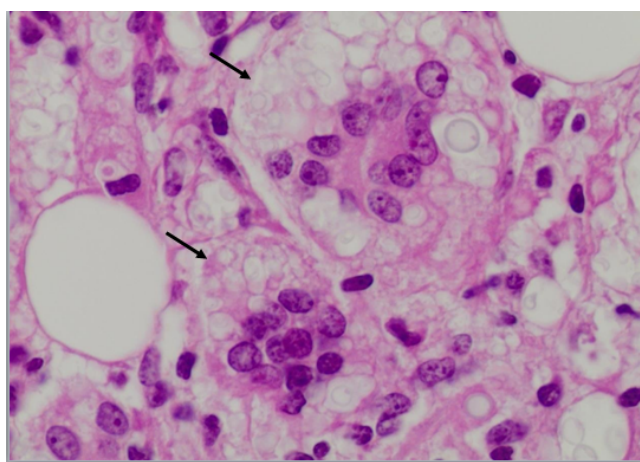


Figure 1: Photomicrograph of bone marrow trephine granuloma. Histiocytes and multinucleated giant cells with centrally aggregated nuclei, comprising the granuloma, are demonstrated. H&E, X50.

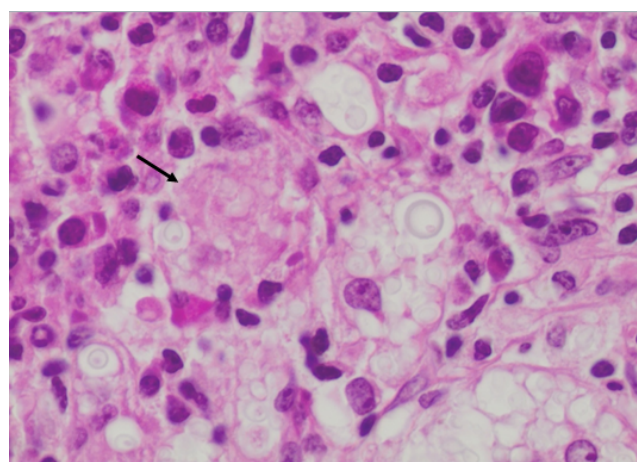


Figure 2: Photomicrograph of bone marrow trephine granuloma. A central area of necrosis within the granuloma is demonstrated. H&E, X50.

The granulomas also demonstrated central areas of necrosis (Figure 2). Numerous phagocytosed, spherical, encapsulated microorganisms surrounded by haloes were observed in clusters within the granulomas as well as scattered interstitially, which were morphologically consistent with *Cryptococcus* (Figure 3).

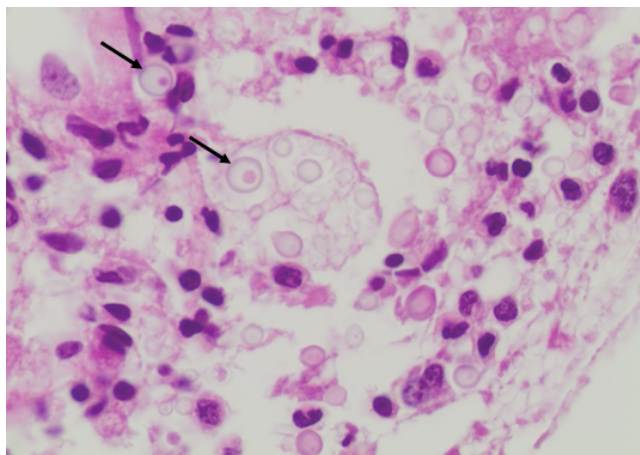


Figure 3: Photomicrograph of bone marrow trephine cryptococci. Numerous phagocytosed, spherical, encapsulated cryptococci are demonstrated. H&E, X50.

Periodic acid-Schiff (PAS) and Grocott's methenamine silver (GMS) stains were performed which highlighted the cryptococci within histiocytes that occurred in aggregates forming the granulomas as well as in single histiocytes interstitially (Figures 4-6).

A Ziehl-Neelsen stain was also performed which excluded acid-fast bacilli.

A diagnosis of disseminated cryptococcal disease of the bone marrow was made, on 11/11/2016, based on morphological and histochemical evidence. The dysplastic changes noted on the aspirate were concluded to be associated with retroviral disease. Subsequently, on 13/11/2016, the patient's blood and CSF fungal cultures were both found to be positive for *Cryptococcus neoformans*.

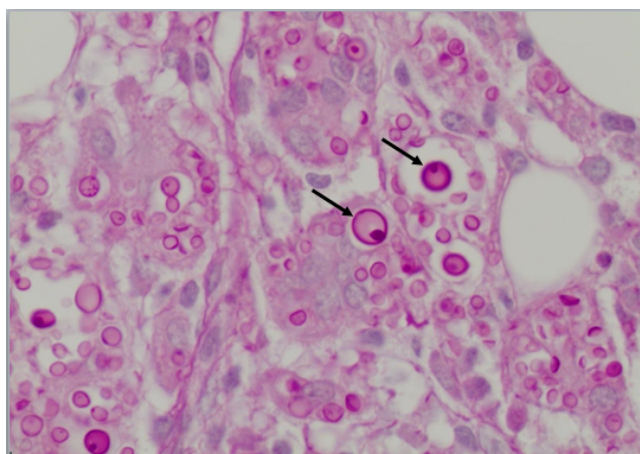


Figure 4: Photomicrograph of bone marrow trephine (PAS stain). The cryptococci are highlighted by staining the thick polysaccharide-rich capsules. PAS, X50.

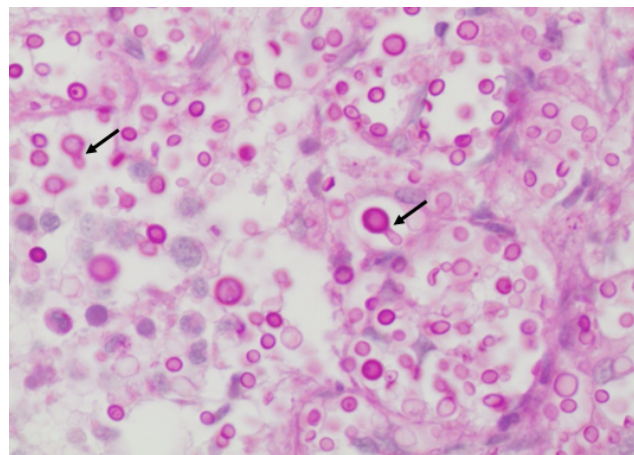


Figure 5: Photomicrograph of bone marrow trephine (PAS stain). The budding cryptococci during replication are highlighted. PAS, X50.

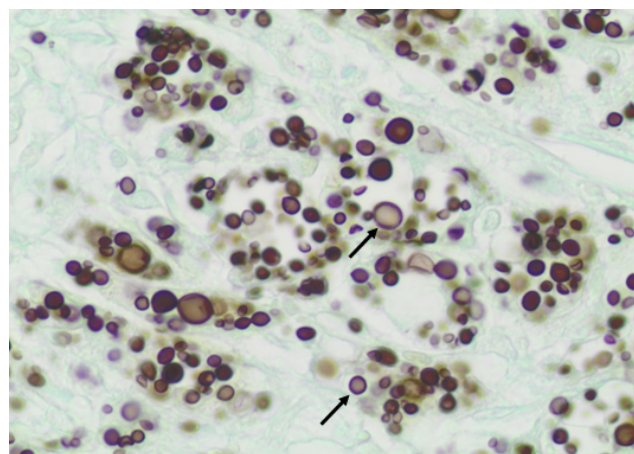


Figure 6: Photomicrograph of bone marrow trephine (GMS stain). The cryptococci are highlighted by staining the capsules. GMS, X50.

The patient commenced treatment with antifungal agents on the day of the bone marrow diagnosis according to the Southern African HIV Clinicians Society guideline 2013 update for cryptococcal meningitis, which included an induction phase of intravenous amphotericin B 50 mg/day+oral fluconazole 800 mg/day for 2 weeks. A repeat fungal blood culture was performed after this induction phase which yielded a negative result. Thereafter, she was discharged and referred to her local ART clinic for continuation of fluconazole as well as ART initiation.

On 09/12/2016, 4 weeks after starting antifungal therapy, the patient was initiated onto first line ART which consisted of tenofovir 300 mg+emtricitabine 200 mg+efavirenz 600 mg as a fixed-dose combination. The antifungal therapy continued with the consolidation phase of oral fluconazole 400 mg/day for 8 weeks and finally a maintenance phase of oral fluconazole 200 mg/day. One year after the start of cryptococcal treatment, on 14/11/2017, the final phase of fluconazole was discontinued after the patient had maintained two consecutive CD4

counts of 264 cells/ μ L and 307 cells/ μ L, taken 6 months apart, with a suppressed HIV viral load. Her full blood count had also completely normalized at this stage.

Discussion

Fungal infections are among the most common opportunistic infections in patients infected with HIV. Cryptococcal disease affects 10-30% of patients with AIDS and causes significant morbidity and mortality [1,2]. Principal sites of cryptococcal disease are the lung and central nervous system [3]. Involvement of the bone marrow is rare [4]. Disseminated cryptococcosis is defined as the demonstration of *Cryptococcus* in blood, sterile body fluids, or tissues other than pulmonary tissue [5].

Cryptococcal disease is caused by two pathogenic species-complexes namely *Cryptococcus neoformans* and *Cryptococcus gattii* both of which collectively comprise of seven clade species [6]. *Cryptococcus neoformans* is an encapsulated yeast that grows and replicates by budding. It is acquired from inhalation of fungal spores or acapsular yeast cells in the environment, particularly from fungi-laden pigeon droppings [3]. Once inside the host, the yeast can change the composition and size of the capsule to increase its chances of resisting or eluding the host's defence mechanisms [7]. Its morphology is characterized by a spherical to oval shape of variable sizes ranging from 5-10 μ m surrounded by a distinct halo and thick capsule; and demonstrates narrow-based budding. PAS and GMS stains allow for better visualisation of these organisms by staining the thick polysaccharide-rich capsules [3].

Cryptococcosis stimulates a granulomatous response in most cases despite immunosuppression. The number of organisms is inversely proportional to the adequacy of the granulomatous response [4]. The granulomatous response is, in turn, dependent on the CD4 count [8]. Cryptococci in the marrow may result in cytopenias by acting in synergy with the HIV; activating macrophages which suppress erythroid progenitor cell development; and by cryptococcal-associated haemophagocytosis [4,9,10].

The Southern African HIV Clinicians Society guideline 2013 update for cryptococcal meningitis recommends that HIV-infected adults with a CD4 count less than 100 cells/ μ L be screened for cryptococcal antigenaemia. This screening may be done by reflex laboratory or clinician-initiated testing. With regards to the latter, screening should be restricted to ART-naïve adults with a CD4 count less than 100 cells/ μ L and no prior cryptococcal meningitis. The screening test, performed on serum or plasma, should be a rapid CrAg test - either latex agglutination test or lateral flow assay. For patients suspected of first episode cryptococcal meningitis, a lumbar puncture should be performed and CSF submitted for a rapid test (preferably CrAg test) as well as a fungal culture [11].

Treatment of cryptococcal meningitis among HIV-infected persons according to the Southern African HIV Clinicians Society guideline 2013 update is divided into three phases: induction phase for 2 weeks with amphotericin B (1 mg/kg/day intravenous)+fluconazole (800 mg/day oral); consolidation phase for 8 weeks with fluconazole (400 mg/day oral); and maintenance phase with fluconazole (200 mg/day oral) for at least a further 10 months until the CD4 count is greater than 200 cells/ μ L for at least 6 months and the most recent HIV viral load is suppressed [11].

Conclusion

Bone marrow examination may be a useful method of diagnosing invasive opportunistic fungal infections such as cryptococcosis as a cause of cytopenias in immunocompromised patients with a negative serum CrAg test, however a CSF CrAg test may have also been useful in this case. This may lead to early diagnosis and treatment thereby decreasing the morbidity and mortality of AIDS patients.

Teaching Points

- Cryptococcal screening with the serum/plasma CrAg test in HIV-infected adults with a CD4 count <100 cells/ μ L and pre-emptive antifungal treatment, for early detection and treatment of cryptococcal disease, is recommended for routine implementation in South Africa.
- For suspected first episode cryptococcal meningitis, CSF should be obtained and submitted for both a CrAg test and fungal culture.
- Bone marrow examination may be a useful method in early diagnosis of disseminated cryptococcosis in immunocompromised patients with cytopenias and a negative serum CrAg test.
- Disseminated cryptococcal disease of the bone marrow stimulates a granulomatous response despite immunosuppression.

The cryptococci in the marrow cause cytopenias by:

- Acting in synergy with the HIV.
- Activating macrophages which suppress erythroid progenitor cell development.
- Cryptococcal-associated haemophagocytosis.

Declarations

Ethics approval and consent to participate

According to the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee-Definition of Health Research and Human Participants Standard Operating Procedure: "Single case reports (≤ 3 patients). Since a single case report does not produce generalizable knowledge it does not meet the definition of research requiring Human Research Ethics Committee approval."

Consent for publication

Written informed consent to publication of a written case report has been obtained from the patient and is available for review by the editors of this journal.

Availability of data and material

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests.

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There are no sources of financial support that facilitated conduct of the work described in this manuscript or the writing of the manuscript itself.

Authors' Contributions

Sumaiya Cassim performed the bone marrow biopsy, collected the data, contributed to histological examination of the bone marrow, and was the major contributor in writing the manuscript.

Monalisa Ntobongwana was a major contributor to histological examination of the bone marrow and making the pathological diagnosis. All authors have read and approved the final manuscript.

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