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Case Report

Speckled-egg staining appearance of cryptococcal osteomyelitis in an immunocompetent patient

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ABSTRACT

Introduction: Cryptococcal infections are typically thought of as occurring in immunocompromised patients, such as patients with HIV/AIDS, solid organ transplant recipients, or patients with rheumatologic diseases that require immunomodulatory therapy. Moreover, *Cryptococcus* spp. classically appear as variably-sized yeasts with narrow-based budding surrounded by a thick polysaccharide capsule. However, cryptococcal infections are being increasingly reported in atypical hosts, at times with non-characteristic histochemical staining appearances. Herein, we report a case of cryptococcal osteomyelitis in an otherwise immunocompetent individual that had a "speckled-egg" staining appearance on direct-smear Gram stain.

Case: The patient is an otherwise healthy 89-year-old male with a past medical history notable only for hypertension who presented with progressive left-sided neck pain that became worse despite lidocaine trigger point injections; imaging was obtained and revealed a C1-C2 prevertebral abscess, C2-C4 osteomyelitis, and a small C2-C4 abscess. An aspiration biopsy from one of the cervical abscesses grew *Cryptococcus neoformans.* Despite prompt initiation of liposomal amphotericin B as soon as the organism was suspected, the patient's mentation declined with associated progression of weakness in his upper and lower extremities. The patient was ultimately transitioned to comfort care.

Conclusions: Unconventional presentations of cryptococcal disease are becoming increasingly recognized in seemingly immunocompetent patients. Our case was unique given that it occurred in a patient who appeared to be immunocompetent and the Gram stain showed a speckled-egg staining pattern that alone was not distinctive for cryptococcal yeasts. Despite the patient's lack of any classic comorbidities associated with invasive cryptococcal disease, his advanced age was likely a risk factor.

Introduction

Cryptococcosis continues to cause a significant burden of disease worldwide. As a result, the World Health Organization (WHO) developed a Fungal Priority Pathogens List (FPPL), published in 2022, that includes both *Cryptococcus neoformans* and *Cryptococcus gattii,* given the continued prevalence and threat that *Cryptococcus* spp. pose [1]. Though cryptococcal infections have historically been well-documented and well-studied in patients with HIV/AIDS, it is becoming increasingly commonplace in other immunocompromised patient populations such as solid organ transplant recipients, hematopoietic stem cell transplant recipients, and patients with rheumatologic diseases that require immunomodulatory therapy [2]. However, what is even more concerning is the growing awareness of invasive cryptococcal disease in otherwise healthy individuals, often presenting with unusual and atypical clinical signs [3]. We report a case of cryptococcal osteomyelitis with a "speckled-egg" staining appearance on direct-smear Gram stain that occurred in an elderly individual who was otherwise immunocompetent. Though granular yeast staining patterns are known to occur on direct-smear Gram staining of specimens containing *Cryptococcus* spp. [4], to the best of our knowledge, this would be the first case specifically detailing the unusual speckled-egg staining appearance that also happened to occur in a patient who would typically be considered immunocompetent.

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Case description

An otherwise healthy 89-year-old male with a past medical history of only hypertension (HTN) initially presented to the emergency department complaining of progressive left-sided neck pain over five days. He reported pain with movement and walking, and denied trauma to the site, numbness or tingling in extremities, shortness of breath, bowel or bladder symptoms, fever, or chills. The patient was subsequently given lidocaine trigger point injections for bilateral supraclavicular tenderness and discharged home. Three days later, he presented again with worsening pain. A cervical spine MRI was obtained and revealed a C1-C2 prevertebral abscess, along with C2-C4 osteomyelitis, which was complicated by a ventral epidural space phlegmon and a small C2-C4 abscess, causing severe spinal cord compression. To further corroborate the diagnosis of osteomyelitis, an ESR and CRP were obtained upon admission that returned as 81 mm/hr and 6.2 mg/dl, respectively. An aspiration biopsy was performed from one of the cervical abscesses, with 8 mL of purulent fluid removed, and was sent to the microbiology laboratory for smear evaluation coupled with bacterial and fungal culture. No material was sent for cytologic or histologic evaluation in Cytopathology or Surgical Pathology, respectively.

A direct-smear Gram stain from the purulent fluid revealed several budding, encapsulated yeast-like organisms with a speckled-egg appearance throughout (Fig. 1). Given concern for possible *Cryptococcus* spp. based on the size and shape of these organisms, the clinical team was contacted directly by Clinical Microbiology and liposomal amphotericin B was initiated. The bacterial and fungal cultures subsequently isolated *Cryptococcus neoformans* and a serum cryptococcal antigen titer was positive at 1:40, but routine blood and AFB/fungal blood cultures did not grow *Cryptococcus neoformans*. Despite the initiation of liposomal amphotericin B as soon as the organism was suspected, the patient's mentation declined with associated weakness of his upper and lower extremities; a lumbar puncture was initially requested but was deferred as he was ultimately transitioned to comfort care due to his worsening overall clinical status. Fungal susceptibilities were not performed.

Of note, the patient's medical history was limited to HTN, although he had previously been assessed for benign prostatic hyperplasia (BPH). He was not on any immunosuppressive medications or steroids, and there was no family history of autoimmune disorders. On admission, the patient's complete blood count with differential showed a total white blood cell count of 9.6 K/cumm, hemoglobin of 12.7 g/dl, hematocrit of 38.1 %, platelet count of 283,000 x 10^9/L, absolute neutrophil count of 8.3 x 10^3cells/μL, absolute lymphocyte count of 0.4 x 10^3cells/μL, absolute monocyte count of 0.8 x 10^3cells/μL, absolute basophil count of 0.1 x 10^3cells/μL, and absolute eosinophil count of 0 x 10^3cells/μL. CD4/CD8 counts and additional immunological testing were not performed because the patient's condition deteriorated quickly during his hospitalization, making further immunological evaluation unfeasible. Aside from his history of hypertension, the patient was in good health and had no other major comorbidities at his advanced age of 89

Discussion

Our case of cryptococcal osteomyelitis was unique for two key reasons; namely, the speckled-egg staining appearance on direct-smear Gram stain, seen in Fig. 1, and the fact that it occurred in an immunocompetent patient. *Cryptococcus* classically appears as a variably-sized yeast, usually 6–7 μm in diameter, though smaller (2–4 μm) and larger (10–20 μm) yeasts can be found within a specimen. Narrow-based budding is typical, though appreciation of this phenomenon can be obscured by the thick polysaccharide capsule, depending on how a specimen was prepared for review. However, capsule-deficient variants of *Cryptococcus* exist [5,6]. In direct specimens, the Gram stain of a *Cryptococcus* species typically shows variably-sized yeasts, with a broad range of staining characteristics including staining strongly purple or strongly pink as well as staining weakly with purple granular inclusions or weakly pink as lipoid bodies [4]. As such, *Cryptococcus* yeasts can be indistinct on Gram staining, even to the seasoned laboratorian, who may question if structures of the right size and shape are actual yeasts or artifact. A well-recognized presentation of *Cryptococcus* yeasts on direct-smear Gram is the observation of yeasts distributed in a crystal-lattice-like pattern as shown in Fig. 2. While we are not aware of explanations of the biochemical underpinnings of this phenomenon in the literature, the crystal-lattice-like pattern plausibly arises due to the physical and chemical forces exerted on the mucinous capsules of adjacent yeasts during the Gram staining process. These factors likely also give rise to the varied staining patterns seen in *Cryptococcus* yeasts, including the speckled-egg appearance that we have noted, which may be a variant of the granular inclusions described by others.

In clinical microbiology, direct specimens can also be stained with Calcofluor-White, which is a fluorescent molecule that non-specifically binds chitin in fungal cell walls. Various stains can be used to visualize *Cryptococcus* in tissue or aspirated material submitted to a Surgical Pathologist or a Cytopathologist, respectively. These include hematoxylin and eosin (H&E), Gomori methenamine silver (GMS), periodic acid-Schiff (PAS), mucicarmine, Alcian blue, and Fontana-Masson stains [5,6]. *Cryptococcus* yeasts do not stain on H&E, but the cell wall may be faintly visible within spaces generated by the similarly non-staining capsule. The cell wall stains on GMS and PAS; whereas the capsule

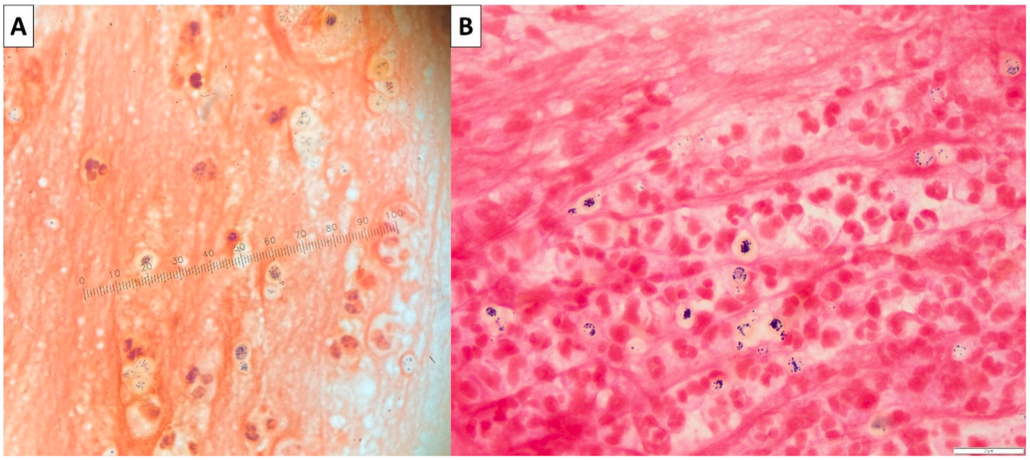

Fig. 1. Speckled-egg appearance of *Cryptococcus neoformans* in a direct smear from the aspiration biopsy. Image collected at 1000x magnification (A), scale bar 20 μm (B).

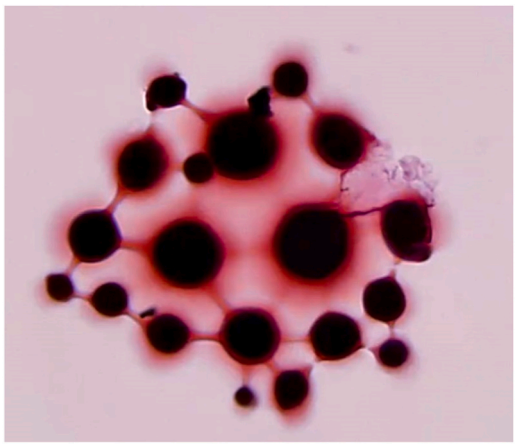

Fig. 2. Crystal-lattice-like presentation of *Cryptococcus* on direct-smear of specimen (CSF) prepared via Gram stain. Image collected at 1000x magnification.

stains via the mucicarmine and Alcian Blue stains. Fontana-Masson is a stain with an affinity for melanin and melanin-like pigments that marks metabolically-active *Cryptococcus* yeasts, though those that are in a lesion with a fibrous capsule (reflecting an organized immune response of some duration) may fail to be highlighted by this stain if they are inert. It is worth noting that all the aforementioned stains are non-specific for *Cryptococcus* and their utility in histologic fungal diagnostics may vary from one histology laboratory to another depending on local protocols. However, Infectious Disease Pathologists may opt to regard yeasts which are highlighted by mucicarmine or Fontana-Masson as definitively identifiable as *Cryptococcus* based on histopathology alone, as long as the GMS or PAS staining profile (size, shape and distribution pattern of yeasts), and the composition of the inflammatory response, is compatible with the diagnosis of *Cryptococcus*. Fig. 3 shows the typical appearance of *Cryptococcus* on histologic review of tissue. The speckled-egg staining appearance noted in this case may be artefactual and reflect local and/or temporal irregularities in the way the Gram stain was performed for this particular specimen, though the staining pattern appeared to be reproducible between machine-based and manual staining techniques. Moreover, neutrophils present in the smear demonstrated the expected staining qualities, tempering this argument. Purely speculatively, the speckled-egg appearance could arise from a confluence of host and pathogen factors pertinent to the biochemistry of the capsule (a known virulence factor), cell wall, and other components of this particular yeast isolate and the character of the local inflammatory milieu. This could be similar to the Splendore-Hoeppli phenomenon seen in the histologic presentation of certain infectious processes such as sporothrichosis or schistosomiasis [7]. Thus, a similar phenomenon in *Cryptococcus*, if found to be present, could serve as a marker of a virulence feature unique to this *Cryptococcus* isolate, enabling it to produce pathology in this immunocompetent host.

Secondly, our case involved a patient who was not

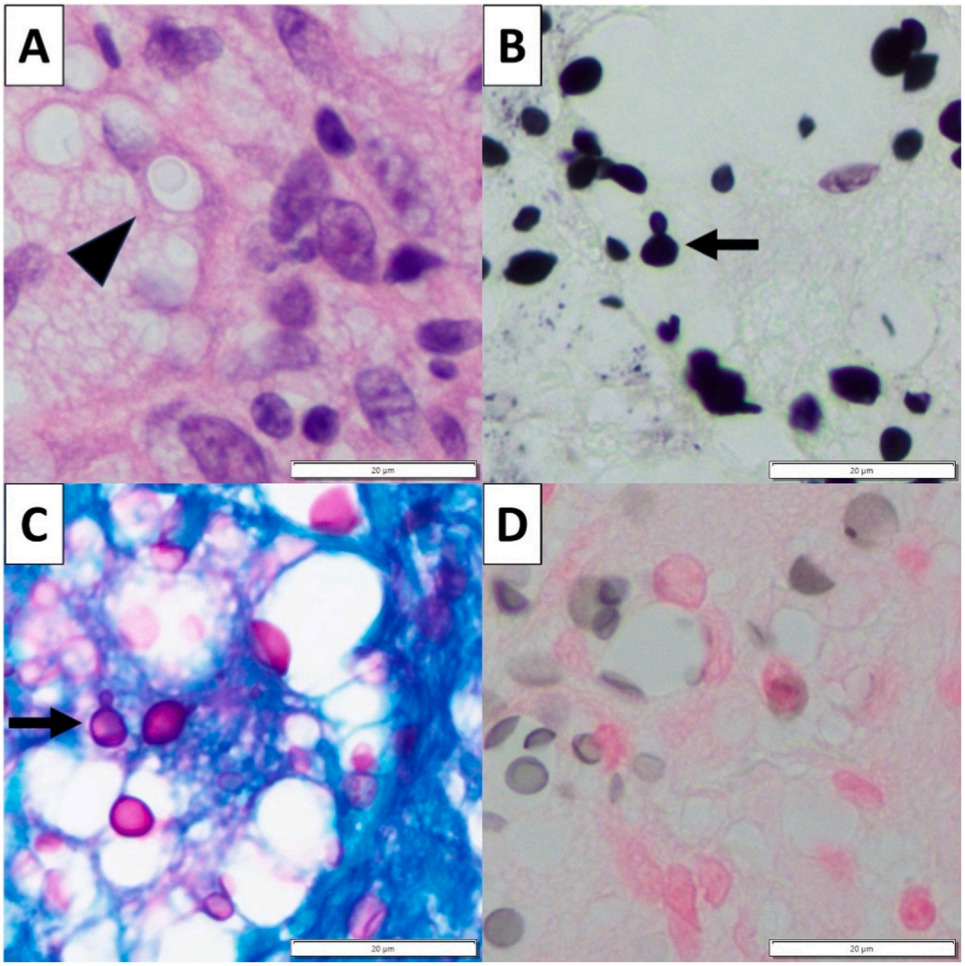

Fig. 3. Characteristic histopathological appearances of *Cryptococcus neoformans* in tissue (skin) via different stains: H&E (A), GMS (B), PAS (C), Fontana-Masson (D). Scale bars are all 20 μm; arrows in B and C indicate narrow-based budding, arrowhead in A indicates non-staining yeast within tissue space generated by non-staining capsule.

immunocompromised; his only medical conditions were hypertension and advanced age. Notably, studies on cryptococcal infections in individuals without HIV or organ transplants have suggested that advanced age may be a risk factor for cryptococcal disease [8–11]. Advanced age is a risk factor not only for cryptococcal infection, but other infections as well due to immunosenescence from an aging immune system [12,13]. This concept helps to provide a biological framework for why advanced age is a risk factor for cryptococcal disease in otherwise immunocompetent individuals. In addition to advanced age, other risk factors for cryptococcal infections in non-HIV, non-transplant patients include (but are not limited to) cirrhosis, cancer, autoimmune/rheumatic diseases requiring immunosuppressive therapy, and chronic heavy alcohol use [8,9,14]. Many healthcare providers may overlook advanced age as a significant risk factor for cryptococcal disease. Therefore, it is essential to raise awareness among clinicians, so they consider *Cryptococcus* in their differential diagnosis, especially in older patients presenting with atypical symptoms.

The importance of including *Cryptococcus* in the differential diagnosis is underscored by the higher mortality rates in older individuals with cryptococcal infections, as they tend to experience worse outcomes [11,15,16]. To further investigate the potential for increased morbidity, mortality, and disease burden in older patients with invasive cryptococcal infections, Tsai et al. studied a cohort of specifically elderly patients with cryptococcal disease and found that elderly patients are more vulnerable to cryptococcal meningitis [17]. Another notable finding from the study was that older patients tend to have more atypical presentations. For instance, headaches, which are typically considered a classic symptom of meningitis, were found to be less common in the elderly group compared to the non-elderly group [17]. In the study by Tsai et al., no other clear risk factors were identified other than those risks that have already been associated with cryptococcal disease in the non-HIV patient group (cirrhosis, cancer, autoimmune/rheumatic diseases requiring immunosuppressive therapy, etc.) [2,8,9,14]. Though the study by Stack et al. looked specifically at young (less than 50 years of age), immunocompetent patients with cryptococcal meningitis, it could likewise not clearly elucidate any other new additional risk factors for invasive cryptococcal disease, other than perhaps a history of illicit drug use [3]. Finally, it is worth noting that in non-HIV patients with cryptococcal disease, it is more likely to manifest outside the central nervous system, as seen in our case [10].

In summary, although *Cryptococcus* may stain uniformly purple or pink in appearance following a Gram stain, with or without a non-staining capsular space, laboratory staff should remain alert of other features including granular inclusions or even a speckled-egg appearance.

Additionally, clinicians should be mindful that cryptococcal infections can occur in patients who are seemingly immunocompetent. Specifically, advanced age is a known risk factor for invasive cryptococcal disease in immunocompetent individuals and many clinicians may not recognize the connection between older age and cryptococcal infection, nor that the symptoms can manifest outside of the central nervous system or lungs.

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CRediT authorship contribution statement

Lavik John-Paul: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Schneider Jack G:** Writing – review & editing, Writing – original draft, Validation, Project administration, Investigation, Data curation, Conceptualization. **Cross Brynne E:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Data curation, Conceptualization. **Stack Matthew Alexander:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization.

Patient consent

Our study was determined to be IRB exempt. We were unable to obtain written consent from that patient because this individual has since passed away. We are prepared to make a good faith effort to contact the patient's next of kin, but we are reluctant to do so as this would unnecessarily disturb the grieving process.

Transparency declarations

Authors BW, MS, JS, and JL declare they have no financial interests or associations that might pose a conflict of interest.

Author Contributions/Statement

BW, MS, JS, and JL jointly conceived the study. BW wrote the first draft of the manuscript and BW and JS performed the chart review, material preparation, and data collection. MS performed the literature review and wrote the second, third, and fourth drafts of the manuscript. JL provided the images for the manuscript. All authors read and approved subsequent versions of the draft (including further revisions of the manuscript after reviewer feedback) in addition to the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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