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CASE REPORT

Cryptococcus Neoformans Osteomyelitis of the Right Ankle Diagnosed by Metagenomic Next-Generation Sequencing in a HIV-Negative Patient with Tuberculous Lymphadenitis and Pulmonary Tuberculosis: A Case Report and Recent Literature Review

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Aim: Cryptococcus neoformans osteomyelitis coupled with tuberculosis and tuberculous lymphadenitis, is a rare occurrence in clinical. Diagnostic challenges arise due to the clinical radiological similarity of this condition to other lung infections and the limited and sensitive nature of traditional approaches. Here, we present a case of co-infection diagnosed using Metagenomic Next-Generation Sequencing, highlighting the effectiveness of advanced genomic techniques in such complex scenarios.

Case Presentation: We present a case of a 67-year-old female infected with cryptococcal osteomyelitis and presented with swelling and pain in the right ankle. Following a biopsy of the right ankle joint, Metagenomic Next-Generation Sequencing (mNGS) of the biopsy tissue revealed Cryptococcus neoformans infection. Positive results for Cryptococcus capsular antigen and pathological findings confirmed the presence of Cryptococcus neoformans. The patient underwent surgical debridement, coupled with oral fluconazole treatment (300mg/day), leading to the resolution of symptoms.

Conclusion: Cryptococcus neoformans is an uncommon cause of ankle infection. Metagenomic Next-Generation Sequencing (mNGS) serves as a valuable diagnostic tool, aiding clinicians in differentiating cryptococcal osteomyelitis from other atypical infections.

Keywords: Cryptococcus neoformans, tuberculosis, diagnosis, metagenomic next-generation sequencing, mNGS

Introduction

Cryptococcosis is an invasive fungal infection caused by Cryptococcus neoformans or Cryptococcus gattii, and its prevalence has been increasing in both immunocompetent and immunocompromised individuals.[1] While cases predominantly affect the central nervous system or the lungs, involvement of multiple other sites remains uncommon. The incidence of bone involvement is approximately 5%.[2,3] Tuberculosis (TB), caused by Mycobacterium tuberculosis bacillus, is a communicable disease that poses a significant global health threat, being a leading cause of illness and mortality. Cryptococcosis and TB are life-threatening infections with a worldwide impact. Distinguishing symptoms, signs, and imaging findings of cryptococcal infection from tuberculosis, tumors, and other bacterial infections is often challenging. Early and accurate diagnosis is crucial for improving the cure rate and prognosis. In this context, we present a case of Cryptococcal osteomyelitis affecting the right ankle in a HIV-seronegative woman with a background of

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pulmonary tuberculosis and lymphadenitis. The diagnosis was established through Metagenomic Next-Generation Sequencing (mNGS) and histopathological examination. Successful treatment involved surgical excision of the lesion and postoperative antifungal and anti-tuberculous therapy. The patient has provided written informed consent for the publication of this case report and accompanying images.

Case Presentation

A 67-year-old female (38.6kg, 153cm) presented with progressive swelling of the right ankle along with pain, leading to decreased mobility of walking and weight-bearing on the right ankle. She reported onset of these symptoms about one month ago, and they have been progressively worsening. The patient had previously been diagnosed with tuberculous lymphadenitis based on cervical lymph node biopsy at another hospital 10 months ago. Additionally, she was diagnosed with secondary pulmonary tuberculosis through bronchoscopic examination at our hospital 5 months ago. Currently, she is undergoing anti-tuberculosis treatment with isoniazid, rifapentine, and levofloxacin, and has received multiple bronchoscopic interventional therapies in our hospital. When her right ankle developed swelling and pain, she initially sought evaluation at a local hospital, where it was considered to be caused by tuberculosis. She was recommended to continue anti-tuberculosis treatment. However, with no significant clinical improvement, she sought further treatment at our hospital.

At the time of presentation, the patient had no cough, sputum production, fever, chills, night sweats, diarrhea, chest pain, dyspnea, palpitations, skin rashes, or neurological symptoms. She had no history of ankle injury or trauma and did not report a history of malignancy, hypertension, diabetes mellitus, cytotoxic therapy, or corticosteroid use. There were no other immunocompromising conditions aside from tuberculosis, and she denied any significant family history. The patient also had no relevant exposures to Cryptococcus, with no history of bird feces or soil contact.

Upon admission to our institution, vital signs were within normal limits: body temperature of 37°C, pulse rate of 80 beats/min, respiration rate of 20 breaths/min, and blood pressure of 124/87 mm Hg. Physical examination at admission revealed increased skin temperature on the surface of the right lateral ankle, noticeable swelling and tenderness, a positive ankle varus trigger test, palpable dorsalis pedis artery, and fine peripheral blood circulation. The remainder of the physical examination, including lung, cardiac, and abdominal examination was normal.

Respiratory, cardiac, and abdominal examinations revealed no abnormalities. Laboratory tests showed a peripheral blood white cell count of 11.9*10^9/L (normal range: 3.5–9.5*10^9/L), with 83.20% neutrophils (normal range: 46.5–76.5%), hemoglobin level of 94 g/L (normal range: 120–170 g/L), platelet count of 416*10^9/L (normal range: 100–300*10^9/L), an elevated erythrocyte sedimentation rate (ESR) of 105.00 mm/h (normal range: 0–20 mm/h), and a C-reactive protein (CRP) level of 57.82 mg/L (normal range: 0–3.3 mg/L). Tumor markers were not elevated, and all other results were normal. Several etiological examinations were conducted including blood culture, sputum culture, pneumococcal antigen test, legionella antigen test, fungi, parasites, mycoplasma test, Chlamydia antigen test, HIV antibodies test, Treponema pallidum test, serum hepatitis A, B, C, E tests, nucleic acid testing for COVID-19, Brucella IgG antibody test, acid-fast stain, and polymerase chain reaction for tuberculosis in sputum, but all results were negative. Chest computed tomography (CT) revealed multiple nodules and cavities in both lung fields (Figure 1). MRI of the ankle joint showed multiple patchy long T1 and long T2 signals beneath the articular surface of the right ankle joint, with the distal end of the fibula as the focal point. The surrounding muscles and subcutaneous soft tissues were significantly swollen, multiple long T1 and long T2 signals were observed within the fibula, and the lesions at the distal end of the fibula were significantly enhanced. Annular lesions were also enhanced, along with the enhancement of cystic lesions in the surrounding soft tissues. The joint space was blurred and narrowed, and the synovial membrane was unevenly thickened and significantly strengthened. Infectious lesions of the right ankle joint with multiple abscesses were evident (Figure 2).

Subsequently, under B-ultrasound guidance, an ankle joint puncture biopsy was performed, yielding a limited tissue sample. mNGS analysis of the puncture specimen revealed the presence of Cryptococcus neoformans. In order to procure sufficient specimens for definitive diagnosis through histological and microbiological examination, the patient underwent surgical intervention involving the complete excision of the lesion. The patient underwent right ankle joint debridement under combined spinal and epidural anesthesia. Intraoperatively, observations included a prominent mass on the right

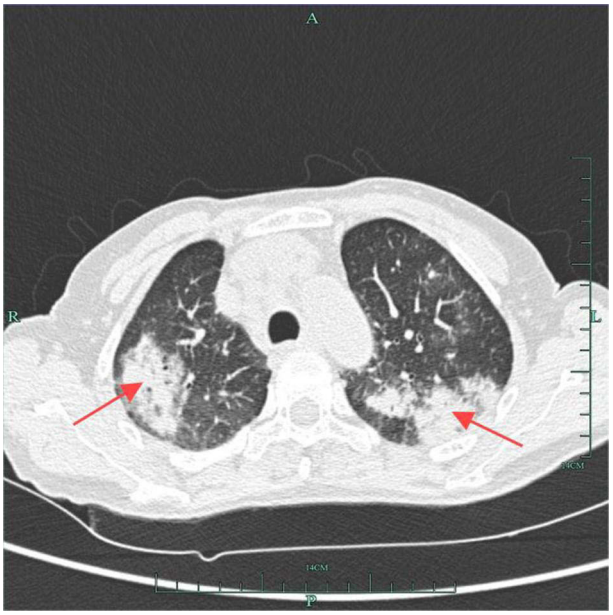

Figure 1 Chest imaging scan at admission, widespread high-density shadows in both lungs. The red arrow indicates the site of the high-density shadow.

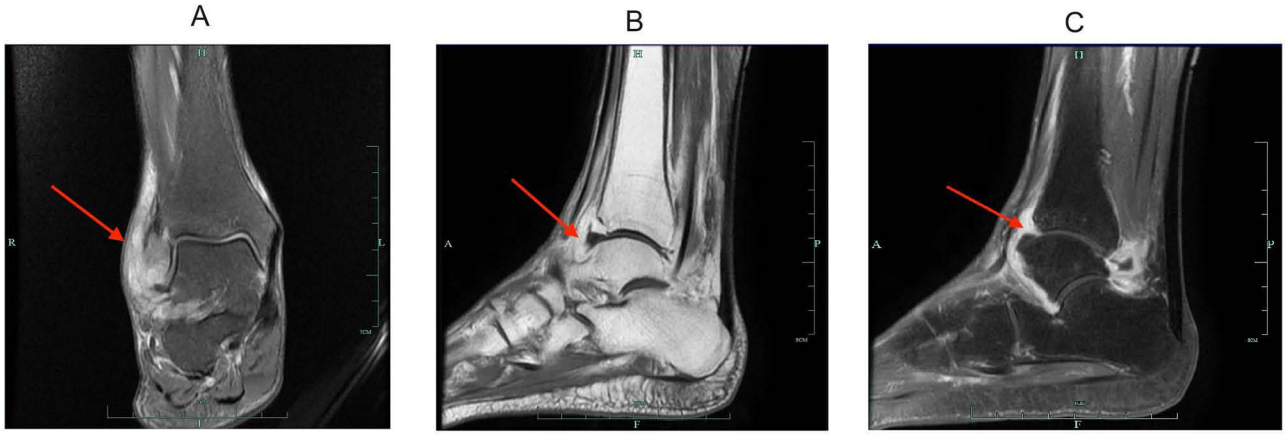

Figure 2 MRI results of right ankle joint on the second day of hospitalization. (**A**) T2WI coronal view of the right ankle joint. The red arrow shows significant soft tissue swelling around the right lateral malleolus. (**B**) T1WI sagittal view of the right ankle joint. The red arrow shows the formation of abscess in the right ankle joint. (**C**) T2WI sagittal view of right ankle joint. The red arrow shows the formation of abscess in the right ankle joint cavity.

lateral malleolus, subcutaneous synovial hyperplasia and necrosis, evident swelling, and the presence of abscesses and moss-like lesions within the lateral malleolar synovial tissue. Additionally, there was notable destruction of the inferior lateral malleolar bone and talonavicular articular surface, along with a greyish discoloration of the synovium. Tissue specimens were collected and subjected to histological, bacterial, fungal, and tuberculosis microbial culture, as well as identification and sensitivity examinations. The returned results were positive for the cryptococcal antigen test, while the remaining tests were negative.

Concurrently, during the surgical procedure, the biopsy tissue underwent pathogen next-generation sequencing at BGI Genomics Co.,Ltd (BGI), reaffirming the presence of Cryptococcus with a relative abundance of 95.16%, encompassing a total of 2781 sequences. Positive results were obtained from both serum and puncture tissue cryptococcal antigen testing.

However, as Cryptococcus was not detected in the multiple bronchoscopic specimens, and the absence of neurological symptoms, the patient declined to undergo lung and lumbar punctures for further assessment of pulmonary and

central nervous system infections. Subsequent to the surgical procedure, the pathological examination revealed the presence of inflammatory granulation tissue with suppuration, along with scattered eosinophil infiltration, and the observation of micro-refractive transparent spheres dispersed in the stroma. Special staining demonstrated: negative antacid staining, positive PAS, PAM, and AB staining (Figure 3). Results from sputum culture, rapid M. tuberculosis culture, and manual M. tuberculosis culture all yielded negative outcomes. Ultimately, the non-HIV patient without underlying diseases was definitively diagnosed with Cryptococcal neoformans ankle osteomyelitis, concomitant with tuberculous lymphadenitis and secondary tuberculosis. Therefore, the treatment regimen of this patient involved a 12-week course of antifungal therapy, consisting of intravenous fluorocytosine (1.0g Q12H for 25 days) and fluconazole (400 mg/day), followed by oral fluconazole (300 mg/day due to gastrointestinal reaction). Concurrently, anti-TB treatment (isoniazid+ rifapentine + levofloxacin) was continued. The patient exhibited improved inflammatory indicators, and the pain progressively alleviated.

Following a 6-month treatment course, chest CT revealed a gradual reduction in lesions, while a MRI scan of the right ankle exhibited significant improvement (Figures 4 and 5). The patient underwent a total of 12 months of antimycobacterial therapy and one and a half years of anti-tuberculosis treatment. The pain progressively diminished, allowing the patient to walk normally. Patient was followed up for 2 years without recurrence.

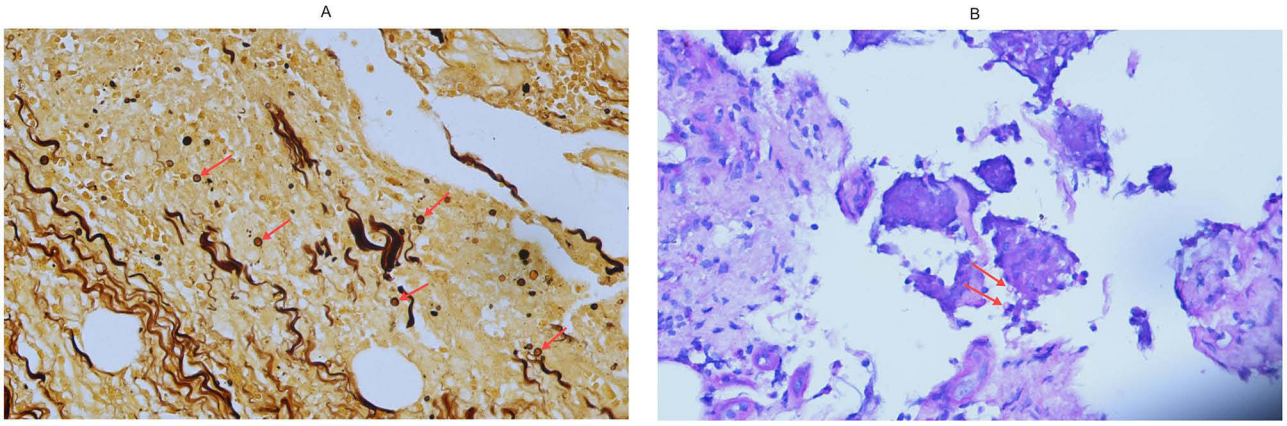

Figure 3 Histopathological staining results of the right ankle joint puncture, scattered microrefraction transparent spherules in the interstitial tissue, suggesting cryptococcal infection. (**A**) Special staining periodic-acid silver methenamine (PAM) staining (×400). Red arrows indicate positive PAM staining. (**B**) Special staining periodic acid–Schiff (PAS) staining (×400). Red arrows indicate positive PAS staining.

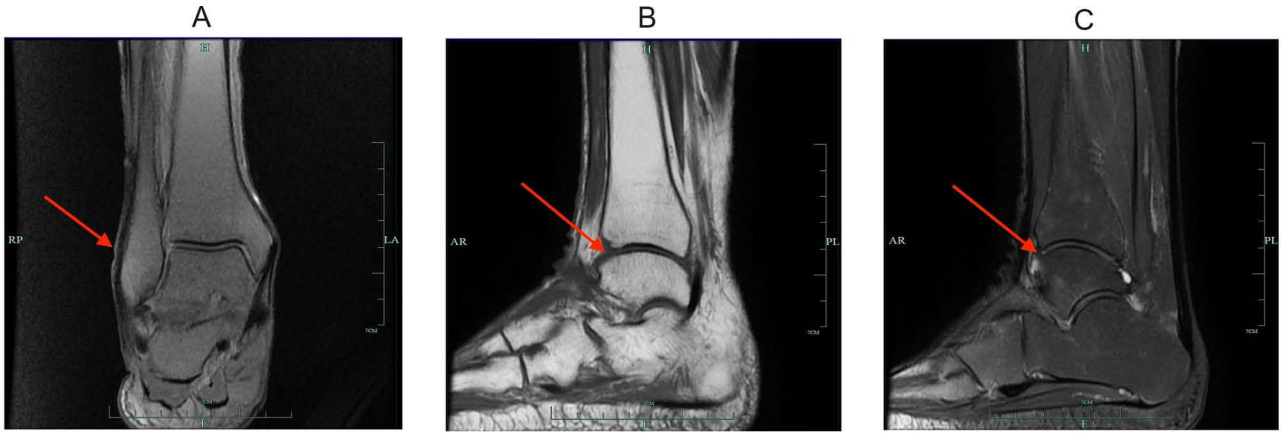

Figure 4 MRI scan of the right ankle after 7 months of oral fluconazole treatment. (**A**) T2WI coronal view of the right ankle joint. The red arrow shows significant soft tissue swelling and absorption around the right lateral ankle. (**B**) T1WI sagittal view of the right ankle joint. The red arrow shows abscess absorption in right ankle joint cavity. (**C**) T2WI sagittal view of right ankle joint. The red arrow shows the absorption of abscess in the right ankle joint cavity.

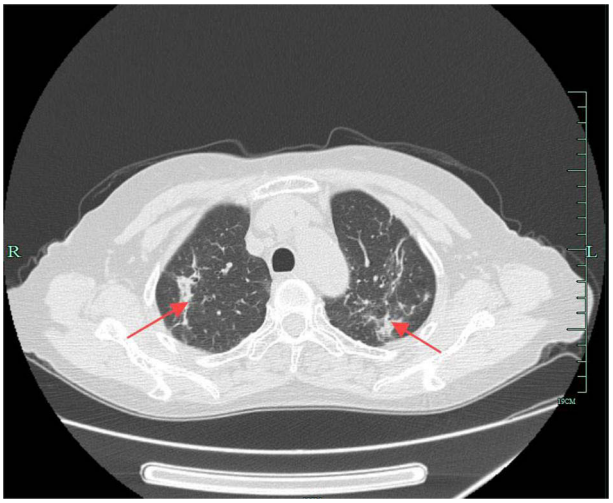

Figure 5 The CT result of the patient's lungs after 9 months of treatment.Red arrows indicate that high-density shadows are significantly absorbed.

Discussion

Cryptococcus neoformans is recognized as an opportunistic pathogenic fungus, capable of causing infections in various parts of the human body.[4] The lungs and central nervous system are particularly susceptible to infection, and other organs or tissues such as the skin, bones, and joints can also be infected.[5–7] The vertebrae, skull, and femur are commonly reported sites of infection[8.] In this case, we present a patient exhibiting a rare and unique manifestation of cryptococcal osteomyelitis in the right ankle. Cryptococcal osteomyelitis in the ankle is an uncommon condition where Cryptococcus invades the bone, accounting for approximately 5% of cryptococcosis cases, particularly in immunocompetent individuals.[9,10]

Both pulmonary tuberculosis and cryptococcosis are life-threatening opportunistic infections that can occur in individuals who appear immunocompetent or severely immunocompromised. This infection is more prevalent in patients with compromised cell-mediated immunity, such as those with conditions like acquired immune deficiency syndrome (AIDS), undergoing hemodialysis, diagnosed with lymphoma or hematologic malignancies, recipients of organ transplantation, individuals with diabetes mellitus, or those receiving corticosteroids or immunosuppressive agents.[11,12]

However, despite their similarities, simultaneous coinfection with Cryptococcus neoformans and Mycobacterium tuberculosis has rarely been reported, and dual tuberculosis/cryptococcosis co-infection is frequently misdiagnosed. The majority of reported coinfection cases involving C. neoformans and tuberculosis typically affect the central nervous system and lungs, respectively.[13,14] In this particular case, tuberculosis infection affected the lymph nodes, while C. neoformans infection involved the ankle, presenting a unique scenario compared to previous reports.

The clinical manifestations and radiological features of Cryptococcal osteomyelitis often lack specificity, posing challenges in distinguishing them from other infectious etiologies, including Staphylococcus aureus, Brucella spp, Actinomyces spp, Mycobacteria spp, or neoplastic processes. This difficulty may lead to delays in diagnosis or misdiagnosis.[15] Notably, tuberculosis is frequently a major cause of misdiagnosis. In this case, the patient presented with concurrent tuberculosis and tuberculous lymphadenitis, further complicating the clinical diagnosis.

Conventional methods commonly employed for diagnosing Cryptococcal osteomyelitis include fungal culture, India ink staining microscopy (India ink), the Cryptococcal capsular polysaccharide antigen (CrAg) test, and histopathology. However, fungal culture often exhibits poor timeliness and low sensitivity. India ink staining microscopy is an economical and rapid method, but it has low sensitivity, and its performance is influenced by the experience of the test performers. The Cryptococcal capsular polysaccharide antigen (CrAg) test currently stands out as the diagnostic assay with the highest sensitivity and specificity, both exceeding 96%. However, it cannot determine the presence of infection, detect antigen-deficient strains, or distinguish specific species.

In recent years, genomics-based microbial detection technology known as Metagenomic Next-Generation Sequencing (mNGS), or high-throughput sequencing, has been increasingly applied in the diagnosis of infectious diseases. This is particularly notable in clinically challenging, critical, and special cases where etiological detection is complex. mNGS demonstrates significant advantages in detecting various infections.[16–18]

This approach is culture-independent and can rapidly detect nearly all known microbes, including bacteria, viruses, fungi, and parasites, in a single run.[19] It excels in identifying strains directly from clinical specimens. In comparison to conventional diagnostic methods, mNGS exhibits slightly lower sensitivity and concordance rates than CrAg tests (97.4%), but higher than those of India ink (63.0%) and culture (76.7%). Additionally, mNGS shows 100.0% sensitivity against culture. Moreover, mNGS can identify Cryptococcus at the species level.[18] Therefore, as a novel etiological diagnostic method, mNGS can be considered a valuable supplementary test for diagnosing Cryptococcal osteomyelitis and directly distinguishing C. gattii from C. neoformans in clinical specimens, contributing to clinical decision-making.

In this case, the primary clinical symptoms exhibited by the patient included right ankle pain, progressive swelling, impaired walking, elevated white blood cell count, and increased erythrocyte sedimentation rate. Imaging findings lacked specificity, necessitating further examination to differentiate them from tumors and tuberculosis. To confirm the diagnosis and alleviate symptoms related to ankle compression, the patient underwent surgery. Ultimately, a precise diagnosis was achieved through timely Metagenomic Next-Generation Sequencing (mNGS) and postoperative histopathology.

The implementation of mNGS in this case shortened the time of diagnosis, enabling guided and accurate medication to reduce the risk of disease progression. Additionally, the hospital stay was shortened, alleviating the economic burden on the patient. Hence, the prompt application of mNGS, especially for patients with severe infections of unknown origin, is recommended.

In the context of cryptococcal infections affecting specific body parts, aside from lung and central nervous system infections, there is no standardized treatment. Published studies suggest that surgical debridement combined with antifungal therapy constitutes a curative treatment for cryptococcal osteomyelitis.[6,20–22]The Infectious Diseases Society of America recommends that, for patients with normal immune status and non-meningeal, non-pulmonary cryptococcosis, oral fluconazole at a dosage of 200 to 400 mg daily for a duration of 6 to 12 months is the primary choice.[23] The treatment proved effective, with all symptoms resolved within 4 weeks. Fluconazole therapy was discontinued after 6 months, and there have been no signs of recurrence as of the 15-month follow-up.

Our patient presented with typical chief complaints and slightly elevated erythrocyte sedimentation rate (ESR) in laboratory data. Despite the absence of systemic signs such as fever and weakness due to local inflammation, a needle biopsy and Metagenomic Next-Generation Sequencing (mNGS) testing were performed to obtain a definitive diagnosis of this very rare disease. The patient underwent surgical debridement in conjunction with antifungal and anti-tuberculosis therapy, leading to the restoration of the ability to walk.

However, there are some limitations to this case report. Examinations for disseminated cryptococcosis should ideally be conducted after identification. Unfortunately, the patient declined a lung biopsy, lumbar puncture for antigen testing and culture, and brain MRI, which could have excluded lung and central nervous system involvement, given the absence of headaches and dizziness.

The CT scan in this case revealed multiple nodules and cavities in both lungs, which are commonly observed in patients with tuberculosis, lung cancer, lung abscess, and mycosis. Ultimately, the absence of central nervous system symptoms throughout the treatment led to the diagnosis of cryptococcal osteomyelitis.

However, it is crucial to acknowledge that Metagenomic Next-Generation Sequencing (mNGS) comes with certain limitations. In less developed areas, particularly in countries with high rates of cryptococcosis but limited resources, this technology may not be readily available in most conventional laboratories. Furthermore, the current cost of mNGS tests is significantly higher than that of conventional diagnostic methods.

Conclusion

In conclusion, ankle osteomyelitis caused by Cryptococcus neoformans is a rare occurrence in HIV-negative patients. Because of the atypical clinical symptoms and imaging findings, Metagenomic Next-Generation Sequencing (mNGS) emerges as a potential diagnostic tool, is important in aiding clinicians in distinguishing Cryptococcus neoformans from

other atypical pulmonary infections. Early intervention is crucial for ensuring prompt diagnosis and treatment, potentially preventing morbidity and mortality. We assert that surgical debridement, coupled with systemic antifungal chemotherapy, represents an effective treatment approach.

Data Sharing Statement

All data used in this study are included in this published article. The sequencing data from mNGS can be searched at the SRA using the accession number PRJNA1136431.

Ethics Approval and Consent to Participate

The ethical approval was acquired by applying for the medical ethics committee of Hangzhou Red Cross Hospital.The patient gave written informed consent to the publication of her history and photographs.

Consent for Publication

Written and informed consent was obtained from the patient for publication of this Case Report and any accompanying images.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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