



Case Report

Challenge of Ziehl-Neelsen stain for Basidiobolomycosis diagnosis in Indonesia: A unique case report

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ABSTRACT

Background: Basidiobolomycosis is a rare fungal infection in Indonesia, so alternative investigations are needed to make a diagnosis.

Case presentation: A 24-year-old male patient, Javanese, presented with Basidiobolomycosis. The patient had a lump on the right arm and face 2 years ago, and the tumor progressively grew up and extended to the right upper arm, neck, and face since 1 month ago. This paper discussed the challenge of Ziehl-Neelsen (ZN) stain as a diagnostic tool for Basidiobolomycosis that is a rare case in the world, especially in Indonesia.

Discussion: Acid-fast stain has never been used to diagnose fungal cases in general, but it could describe the hyphae and Splendore-Hoespli very clearly compared to the standard hematoxylin and eosin (HE) stains.

Conclusion: ZN can be used as an alternative diagnosis of Basidiobolomycosis in a low-resource setting.

1. Introduction

Basidiobolomycosis is a rare fungal infection in Indonesia that is characterized by the formation of fluctuant arm and non-tender swellings, generally on the extremities and trunk in the immunocompetent subject. Basidiobolomycosis is a granulomatous type of skin and subcutaneous tissues caused by *Basidiobolus ranarum*. Definitive diagnosis is microscopy and histopathology examination. Acid-fast staining has never been used to diagnose mold in general. This staining is widely known to identify acid-fast organisms, especially *Mycobacterium* and other organisms such as *Actinomyces* spp. [1,2].

Fast acid bacilli, such as *Mycobacterium tuberculosis*, contain large amounts of mycolic acid in the cell wall, which is resistant to common stains such as Gram stain [1]. Ziehl-Neelsen (ZN) reagent consists of carbol fuchsin, acid alcohol, and methylene blue. Carbol fuchsin gives each cell a red color, which is removed by acid-alcohol. The organism is not acid-fast, does not have a thick lipid layer that can withstand carbol fuchsin so that when a counterstain (methylene blue) is exposed, the organism will pick it up so that the color turns blue under a microscope. Acid-fast organisms retain carbol fuchsin so that it looks red [3]. Based on the description above, this study reported a unique case of ZN staining on Basidiobolomycosis in Indonesian adults. We report base on

Surgical Case Report (SCARE) 2020 guideline [4].

2. Case presentation

A 24-year-old male patient presented a lump on the right arm and face 2 years ago. The solid and hard lump with no pain and itchy started from the right arm. The patient had received a biopsy and was given anti-tuberculosis therapy for 6 months but no improvement. The tumor progressively grew up and extended to the right upper arm, neck, and face 1 month ago. The patient was diagnosed with suspected soft tissue sarcoma of the right superior extremity region T4NxM0 and lymphedema. The histopathology examination showed granulomatous inflammation with multinucleated giant cells. ZN stain showed no acid-fast bacilli, but hyphae and Splendore-Hoespli phenomenon (Fig. 1). The patient was then treated according to the diagnosis of Basidiobolomycosis with Itraconazole for 1 year and recovered completely.

We confirmed this finding by performing the usual hematoxylin and eosin (HE) stain for histopathological examination of fungal infections. The results of HE staining showed multinucleated giant cells with hyphae accompanied by the Splendore-Hoespli phenomenon (asteroid bodies; Fig. 2). The patient received itraconazole therapy 2 × 100mg/day after the patient confirmed Basidiobolomycosis. After 1 year of

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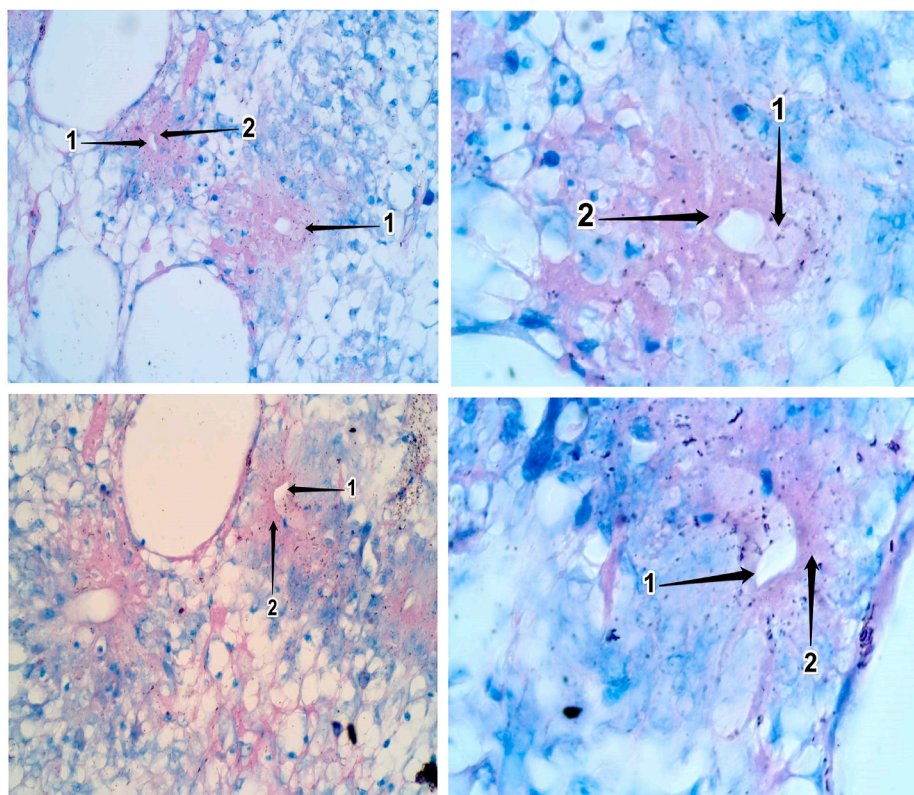


Fig. 1. Hyphae (1) with Splendore-Hoeppli phenomenon (asteroid bodies) (2) in acid-fast staining [ZN stain 400×, 1000×].

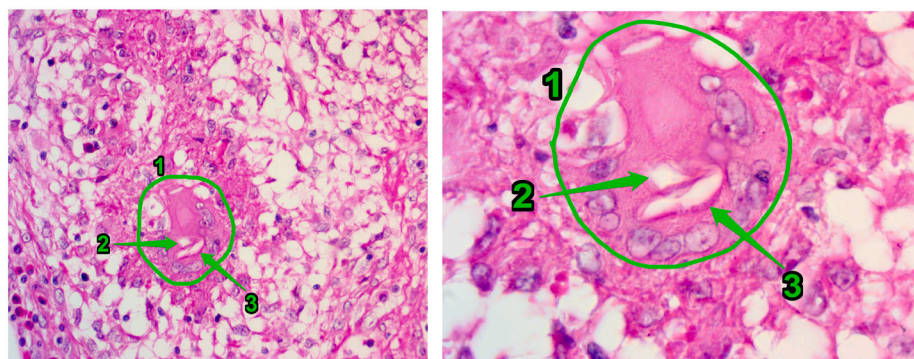


Fig. 2. Multinucleated giant cell (1); Hyphae (2) with Splendore-Hoeppli phenomenon (asteroid bodies) (3) surrounded by radiating intensely eosinophilic material that to be generated by a fusion of necrotic cells and host inflammatory cell [HE 400×, 1000×].

therapy, the patient experienced improvement.

3. Discussion

Acid-fast staining has never been used for identification staining of *Basidiobolus* spp. Ziehl–Neelsen stain is a type of narrow-spectrum fungal stain that selectively differentiates and identifies fungi so that this stain is rarely used in diagnosing suspected fungal infections, especially in Indonesia [5]. Several types of fungi that are generally acid-fast and usually stained with ZN stain are *Histoplasma* and *Russula* spp [6,7]. In this case, we found a Splendore-Hoeppli picture on ZN stain with a clinical picture of suspected Basidiobolomycosis and microbiological examination of no fungi growth.

The Splendore-Hoeppli phenomenon is the in vivo formation of highly eosinophilic (club-shaped or band-like eosinophilic structures) surrounding microorganisms (fungi, bacteria, and parasites) with HE

staining [8]. The Splendore-Hoeppli phenomenon in fungal infections can be seen in sporotrichosis, pityrosporum folliculitis, zygomycosis, candidiasis, aspergillosis, and blastomycosis. The Splendore-Hoeppli phenomenon in Basidiobolomycosis includes zygomycosis in the form of epithelioid cell granuloma (multinucleated giant cell) with fungal hyphae accompanied by eosinophilic infiltration [9]. This eosinophilic material is composed of antigen-antibody complexes, tissue debris, and fibrin. This reaction is not known with certainty and is thought to be a local immune response to the deposition of antigen-antibody associated with fungi, parasites, bacteria, or inert materials, which may be an attempt by the body (host) to retain the harmful agent. However, it may also function to prevent phagocytosis and intracellular death by agents causing prolonged infection [10]. The Splendore-Hoeppli structure also represents glycoproteins, lipids, and calcium derived from host leukocytes [11].

The positivity of Splendore-Hoeppli, in this case, maybe due to acid-

fast eosinophilic basidiobolus material, both related to basidiobolus organisms, antigen-antibody complexes, tissue debris, and fibrin as well as glycoproteins, calcium lipids derived from host leukocytes that accumulate during infection, but it requires further study because the mechanism of the Splendore-Hoeppli reaction itself is not fully known. However, it is thought that at least two mechanisms are involved in the formation of the Splendore-Hoeppli material, and the components may differ according to the infecting organism and the immunoreactive cells. Researchers need to consider the level of immunocompetence and allergy in each host, as well as the fact that organisms vary greatly in characteristics such as form, toxicity, and pathogenicity, as well as Splendore-Hoeppli materials resulting from host-parasite interactions which may be heterogeneous [11]. diagnosis of Basidiobolomycosis is often found negative on fungal cultures [12,13].

Management of Basidiobolomycosis infection has been using itraconazole 2×100 mg/day for 3 months. In some cases, if itraconazole has a good response, it can be continued for several months. In our case, it was reported that itraconazole was administered for ± 1 year [14,15].

4. Conclusion

Acid-fast staining has never been used for staining identification of *Basidiobolus* spp. However, the authors find that ZN stain can detect hyphae and Splendore-Hoeppli in Basidiobolomycosis. This occurs because of the immunological response in each host, and variations in characteristics such as form, toxicity, and pathogenicity of organisms and Splendore-Hoeppli materials resulting from heterogeneous host-parasite interactions. This condition requires further research with an adequate number of samples.

Ethical approval

We have conducted an ethical approval based on the Declaration of Helsinki with registration research at the Health Research Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Provenance and peer review

Not commissioned, externally peer-reviewed

Registration of research

Name of the registry: -

Unique identifying number or registration: -

Hyperlink to your specific registration (must be publicly accessible and will be checked): -

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Guarantor

Arthur Pohan Kawilarang.

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Author's contribution

All authors contributed toward data analysis, drafting, and revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.amsu.2022.103278>.

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