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Case Study

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Detection of Antibody-Coated Mucor in Skin Biopsy by Direct Immunofluorescence

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Abstract

Cutaneous mucormycosis may be caused by direct inoculation or hematogenous spread of mucormycetes in immunocompromised patients. Skin biopsy is characterized by a deep fungal infection with frequent angioinvasion. The fungal hyphae can usually be identified on hematoxylin-eosin stain. We report a case of cutaneous angioinvasive mucormycosis in which the fungi were also visualized on direct immunofluorescence. A 57-year-old patient with relapsed myelodysplastic syndrome status-post allogeneic hematopoietic cell transplant, diabetes mellitus, and graft-versus-host disease presented with painful, palpable, dark red to violaceous retiform purpuric plaques. Light microscopy of punch biopsy revealed numerous broad, ribbon-like, pauci-septate hyphae in the dermis with angioinvasion, consistent with mucormycosis. Direct immunofluorescence performed on a concurrent biopsy to exclude immune complex vasculitis demonstrated smooth IgG, IgA (weak), IgM (faint), and C3 deposition on the hyphal structures, compatible with antibody-coated fungi. Tissue culture subsequently confirmed *Mucor* species. Although mucormycosis was readily diagnosable on routine light microscopy in this case, recognition of the unique phenomenon of antibody-coated fungi can be crucial when the invasive fungi are sparse or only present in the direct immunofluorescence specimen.

Key words: Cutaneous mucormycosis, direct immunofluorescence, antibody-coated fungi

Introduction

Mucormycosis is a rare disease caused by fungi of the order Mucorales, most commonly *Rhizopus* and *Mucor* species. Increased incidence has been reported during the COVID-19 pandemic, most notably in South Asia.[1-3] Mucormycosis carries a high mortality rate and primarily affects immunocompromised patients or those with poorly controlled diabetes. Patients may present with cutaneous, pulmonary, rhinocerebral, gastrointestinal, or disseminated disease.[4-7] Cutaneous mucormycosis may be a result of direct inoculation at a site of trauma or hematogenous spread of mucormycetes. The clinical presentation of cutaneous mucormycosis varies but is usually characterized by tender plaques or necrotic eschars with surrounding erythema and induration.[8-10] Deep tissue extension or dissemination is common. As the clinical morphology may be nonspecific, high clinical suspicion is paramount for early treatment initiation.[11]

While fungal culture is one way to confirm the diagnosis, its turnaround time is inferior to that of histopathologic evaluation of either frozen or permanent tissue sections.[12, 13] Broad, ribbon-like, pauci-septate fungal hyphae can usually be identified on hematoxylin-eosin (H&E) stain. However, when the organisms are sparse or obscured by inflammation, thrombosis, or necrosis,[4, 6, 9, 10] periodic acid-Schiff (PAS) and Grocott methenamine silver (GMS) stains may be needed to highlight the hyphae. Because fragmented and necrotic fungal elements in mucormycosis may give rise to falsely negative staining on GMS stain, PAS is generally preferred.[13] In this report, we describe visualization of *Mucor* hyphae on direct immunofluorescence study.

Case Report

A 57-year-old man with type 2 diabetes mellitus and myelodysplastic syndrome (MDS) status-post allogeneic hematopoietic cell transplant was admitted for severe thrombocytopenia, rash, and increasing liver function tests, concerning for graft-versus-host disease (GVHD) of the skin and liver. Blood work at admission was remarkable for neutropenia (absolute neutrophil count 0.4 K/cmm; normal range 1.5-7.2 K/cmm). Due to concern for GVHD, he was treated with methylprednisolone, ruxolitinib, anti-thymocyte globulin, and etanercept. Prior to anti-thymocyte globulin treatment, flow cytometry showed a reduced T-cell population (30.9% CD3 positive T cells; normal range 61-79%). However, following anti-thymocyte globulin treatment, flow cytometry of peripheral blood revealed severe T-cell depletion (0% CD3 positive T cells), while B-cell population remained within normal limits.

On day 113 status-post hematopoietic cell transplant, he developed painful and palpable dark red to violaceous retiform purpuric plaques on the abdomen associated with insulin injection sites, and similar purpuric lesions on the upper and lower extremities unassociated with trauma (Fig 1). Dermatology was consulted, and there was high clinical suspicion for an angioinvasive fungal infection given the patient's neutropenic state and history of diabetes. Other clinical considerations included other infections, medium vessel vasculitis, and vasculopathy.

Punch biopsy of a representative skin lesion on the left medial thigh revealed numerous ribbon-like, pauci-septate, angioinvasive hyphae in the dermis consistent with mucormycosis (Fig 2). The involved vessels were frequently thrombotic, resulting in "*Mucor* thrombi". The organisms were highlighted by both PAS and GMS stains. Direct immunofluorescence performed on a concurrent biopsy to exclude immune complex vasculitis demonstrated smooth IgG, IgA (weak), IgM (faint), and C3 deposition on numerous ribbon-like hyphal structures in the dermis, compatible with coating of mucormycetes with these immunoglobulins and complement (Fig 3).

No organisms were observed on the fibrinogen-stained slide or the negative control, and no vascular immune deposits were identified. Tissue culture subsequently confirmed *Mucor* species.

Intravenous amphotericin B was initiated for disseminated mucormycosis. He also developed vancomycin-resistant enterococcus bacteremia, which was treated with daptomycin, and pneumonia treated with cefepime. He continued to be transfusion dependent with worsening GVHD, progression of MDS, and persistent disseminated mucormycosis. Ultimately, he was transitioned to home hospice and expired shortly thereafter.

Discussion

The patient in this case carried multiple risk factors for mucormycosis, including hematologic malignancy, hematopoietic cell transplantation, GVHD, severe immunosuppression, and diabetes mellitus. A traumatized skin barrier, in this case via insulin injection, is required for primary cutaneous mucormycosis to develop. The angioinvasive characteristic of mucormycetes may then lead to hematogenous dissemination to other sites.[14]

Our patient was treated with a combination of methylprednisolone, ruxolitinib, anti-thymocyte globulin, and etanercept for GVHD. Both GVHD and these immunosuppressive drugs together confer severe neutropenia and T-cell depletion, resulting in an increased risk of infections.[15-18] From both clinical and experimental data, impaired phagocytosis due to neutropenia seems to be a primary driving factor in the pathogenesis of mucormycosis. Although T-cell depletion alone does not lead to increased fungal spore proliferation, it is likely that both intact neutrophil and T-cell responses are necessary to effectively clear the invasive fungi.[14] In an immunocompetent host, fungal cell wall components activate antigen-presenting cells, causing the release of various

cytokines. Naïve T-cells are exposed to these cytokines and are prompted to differentiate into T_H17 cells. T_H17 cells produce IL-17A and IL-22, which lead to the production of proinflammatory cytokines, chemokines, and antimicrobial peptides, ultimately recruiting neutrophils to the infection sites to destroy the fungal organism.[19, 20] In an immunocompromised host such as our patient, both the T-cell and the neutrophil responses are impaired, significantly limiting the host's ability to clear the fungi. However, the immune response in our patient was not completely ablated, as evidenced by the direct immunofluorescence findings of host immunoglobulins and C3 deposition on the fungal hyphae. Flow cytometry showed the B-cell population was within normal range, suggesting preserved antibody production via the B-cell lineage. During infection, host antibodies are expected to bind to foreign proteins, prompting phagocytosis, complement-mediated lysis, and other immune responses.[21]

Literature on antibody-coated microorganisms is largely limited to the examination of urinary sediments by direct immunofluorescence, in which a positive result would indicate local production of antibodies in the kidney and support a diagnosis of bacterial or fungal pyelonephritis over a lower urinary tract infection. The urinary pathogens were most frequently coated with IgG and IgA antibodies, similar to the observations in our case.[22-24] Our case also demonstrated strong C3 deposition in the same pattern as the immunoglobulins. C3 is a complement protein which primarily acts to facilitate immunologic pathogen clearance via increased uptake by phagocytic cells and activation of the adaptive immune response. C3 protein is primarily produced in the liver and distributed via serum.[25] Like its action in bacterial infections, the complement cascade has been well documented in antifungal immunity, with C3 rapidly depositing on fungal surfaces to facilitate clearance.[26-29]

Interestingly, autofluorescence of fungal organisms has been well reported when viewing H&E-stained slides prepared from formalin-fixed and paraffin-embedded tissues under a fluorescence microscope.[30-32] In our case, however, the direct immunofluorescence sample was not formalin-fixed or H&E-stained, and the absence of immune deposits on the negative control and fibrinogen-stained slide speaks against autofluorescence as the cause of our findings.

A potential diagnostic pitfall is the misinterpretation of fungal cell wall staining on direct immunofluorescence as vascular immune deposition. Although vasculitis typically demonstrates granular immune deposits in the vessel walls, smooth vascular deposits may be seen in porphyrias,[33] bullosis diabeticorum,[34] and possibly other conditions. Careful attention to the morphology (shape and size) of the cell walls and the background blood vessels is required to distinguish between the two. Correlation with the findings on H&E and PAS stains would also be important.

Based on our finding of antibody-coated fungi on direct immunofluorescence, we hypothesize that in neutropenic and T-cell depleted patients, the intact humoral immune system may allow for recognition and opsonization of cutaneous pathogens, but the impaired cell-mediated response fails to complete the next steps in pathogen clearance. Although mucormycosis is readily diagnosable on routine light microscopy in most cases, recognition of the phenomenon of antibody-coated fungi can be crucial in making an accurate and timely diagnosis when the invasive fungi are sparse or only present in the specimen submitted for direct immunofluorescence.

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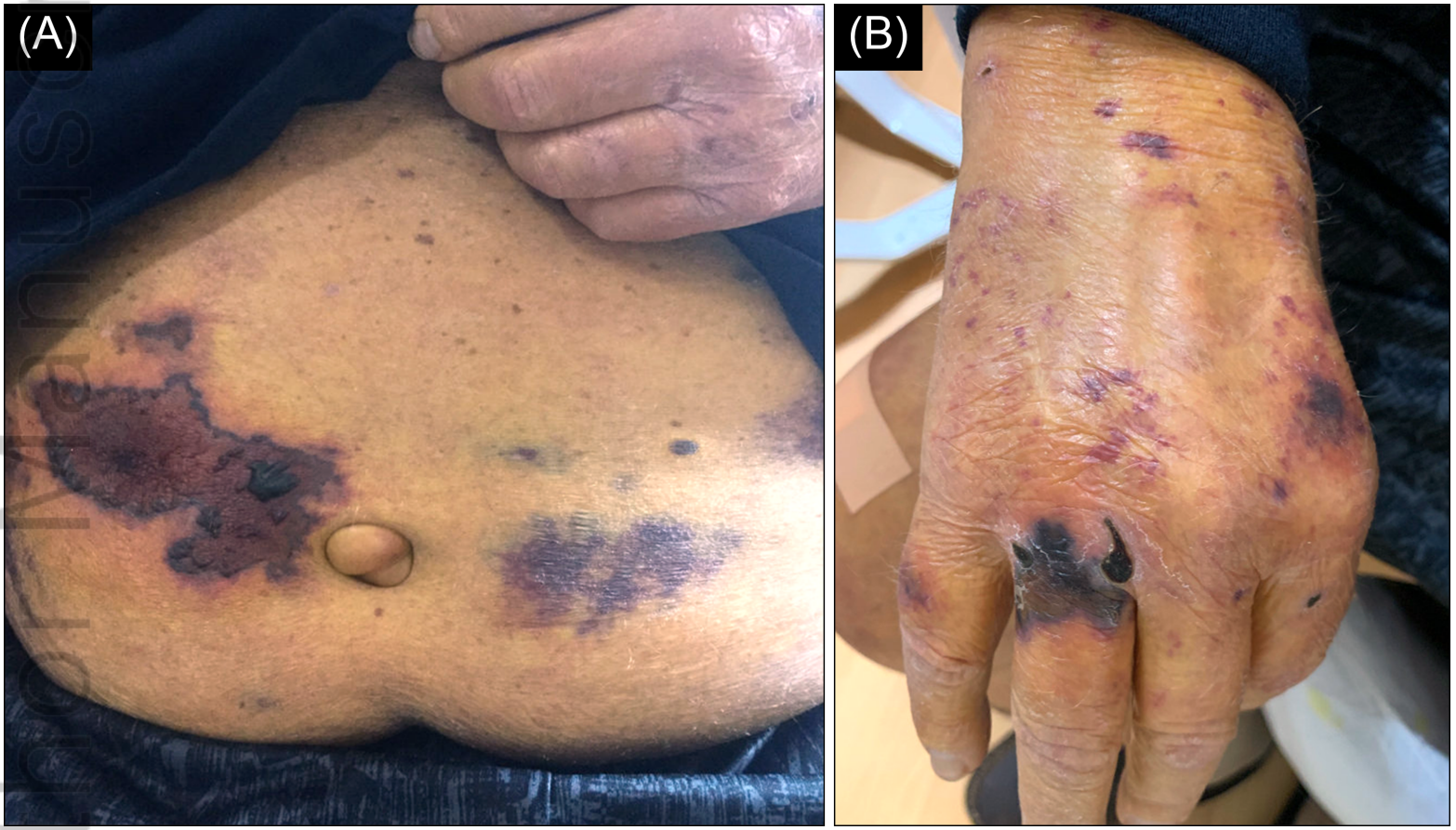
Figure Legends

Fig 1. Clinical morphology of representative skin lesions. A) Retiform purpura and a large violaceous plaque with an erythematous rim and necrotic-appearing bullae were present on the

abdomen, corresponding to the insulin injection sites. B) Multiple violaceous and necrotic-appearing patches and plaques were present on the right hand.

Fig 2. Punch biopsy of a skin lesion on the left medial thigh. A) Scanning magnification reveals extensive dermal hemorrhage. B) Numerous broad, ribbon-like, pauci-septate hyphae are present in the dermis, associated with acute inflammation. C) There is frequent angioinvasion by these organisms, resulting in necrosis and thrombosis of the vessels. (Hematoxylin and eosin stain; Original magnifications ×20 [A], ×400 [B and C])

Fig 3. Direct immunofluorescence study of skin biopsy. A, B) Numerous ribbon-like hyphal structures are highlighted by IgG. C) A smaller number of hyphae are highlighted by IgA. D) Faint IgM deposition is observed on a few hyphae. E, F) Many hyphae display strong C3 deposition. (Original magnifications ×200 [A], ×400 [B-D, F], ×100 [E])



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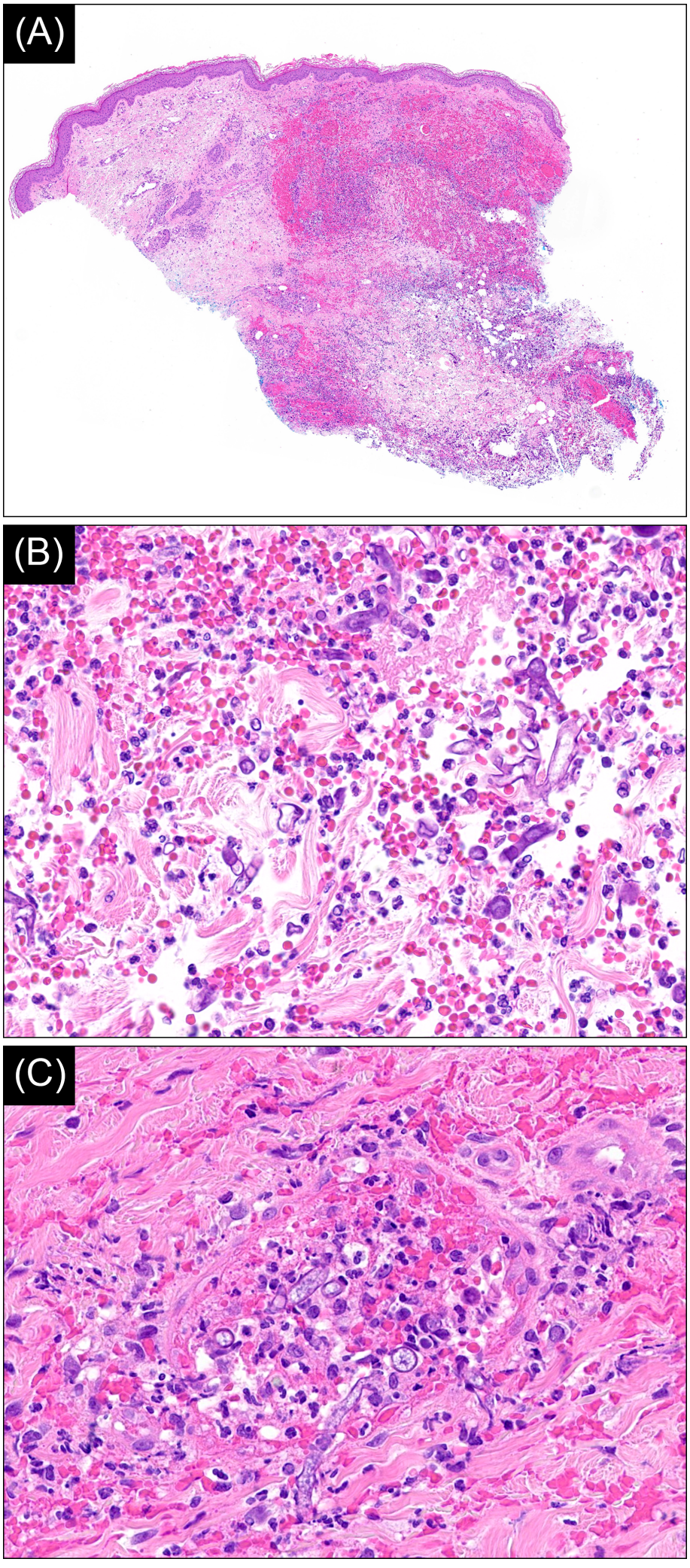

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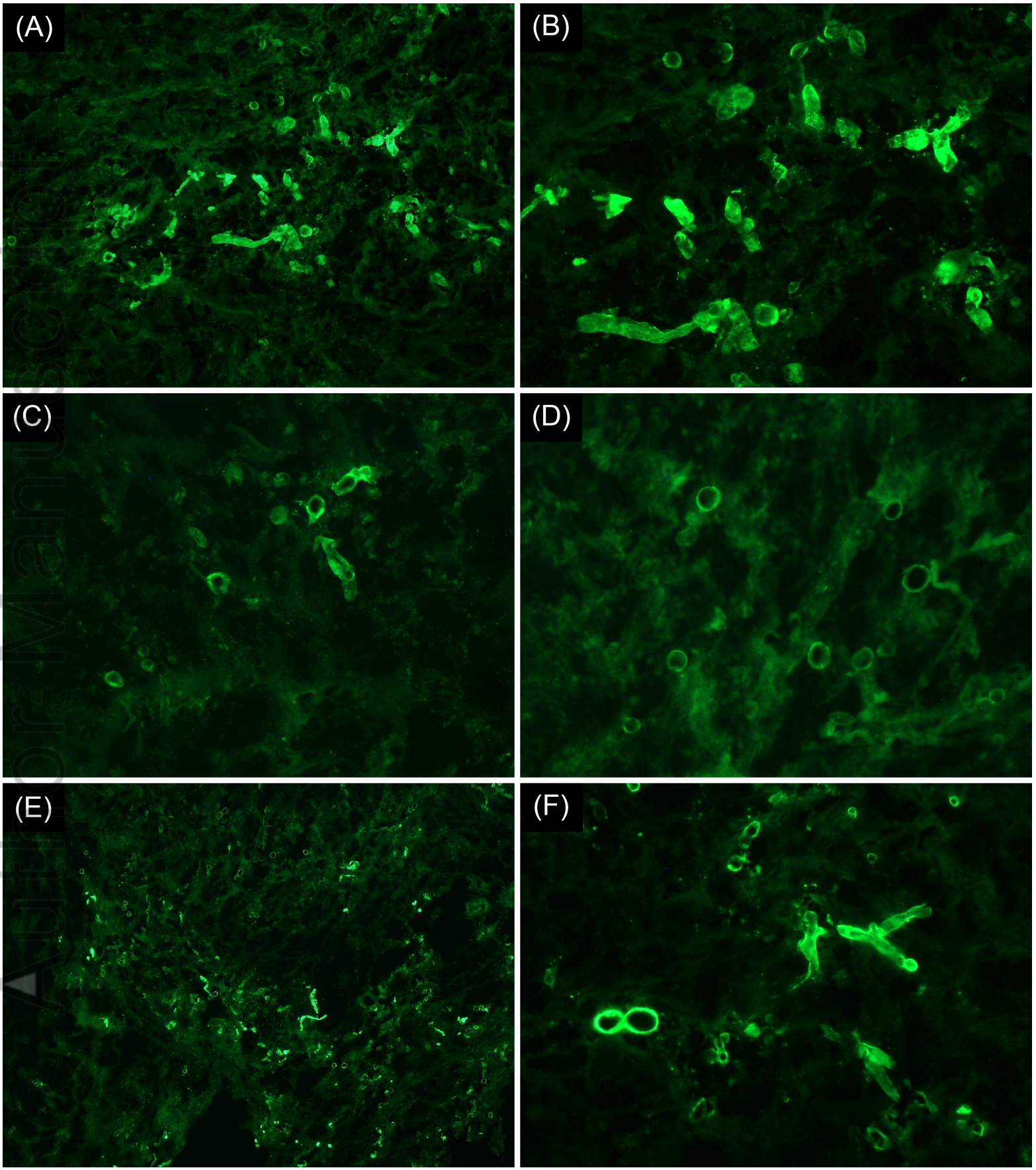

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