Case Presentation

# Complement fragment 4d staining as a potential diagnostic tool for bullous disorders with esophageal involvement

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## Abstract

Mucous membrane pemphigoid (MMP) is an autoimmune blistering disorder that can involve the esophagus, potentially leading to complications such as stricture or stenosis. Diagnosis of MMP is challenging owing to overlapping clinical features with other subepithelial blistering diseases and limitations of current diagnostic techniques. Although direct immunofluorescence (DIF) remains the gold standard, it requires fresh tissue samples, which are not always available, particularly in cases without active mucosal or cutaneous lesions. Complement fragment 4d (C4d) immunohistochemistry (IHC) on formalin-fixed tissue has demonstrated diagnostic utility in bullous pemphigoid but has been less explored in MMP. This case highlights that C4d IHC may serve as a useful ancillary test to support MMP diagnosis, especially for mucosal or esophageal biopsies received in formalin, offering a potential diagnostic pathway when fresh biopsy samples are unavailable.

## Introduction

Mucous membrane pemphigoid (MMP) is a blistering disorder caused by autoimmune reactions targeting basement membrane proteins, resulting in subepithelial blistering predominantly in mucous membranes. MMP can be suspected clinically but requires pathological confirmation.[1-4] Direct immunofluorescence (DIF) remains the gold standard for MMP diagnosis; however, it requires a non–formalin-fixed fresh tissue specimen.[1-5] Other diagnostic methods, including indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assays (ELISAs), can assist in MMP diagnosis without the need for fresh tissue for DIF but are limited in sensitivity and specificity.[1-3,5] Complement fragment 4d (C4d) immunohistochemistry (IHC) on formalin-fixed paraffin-embedded tissue has been proposed as a sensitive approach for diagnosing bullous pemphigoid (BP),[2,3,5-7] although its use in mucosal and esophageal biopsies remains underexplored.[1,3,4] In cases of bullous disorders with primarily oral or esophageal involvement, formalin-fixed mucosal biopsies may be more readily available. We report the use of C4d IHC to retroactively support the diagnosis of anti-BP antigen MMP in a patient with a previously nonspecific formalin-fixed esophageal biopsy, in the absence of active oral or cutaneous lesions.

## Case Synopsis

A 69-year-old woman presented with a 4-year history of recurrent oral and genital erosions, initially diagnosed as Behçet's disease. Colchicine 0.6 mg twice daily and valacyclovir 500 mg daily resolved the genital lesions but did not improve the oral lesions. The patient was hospitalized for dysphagia, odynophagia, and chest pain, during which a formalin-fixed biopsy obtained via esophagogastroduodenoscopy demonstrated active esophagitis with ulceration and was negative for herpes simplex virus immunostaining (**Figure 1**).

Antibody testing by IIF showed negative IgG paraneoplastic pemphigus antibodies to rat bladder, mouse bladder, mouse heart, and mouse liver substrates. A concurrent serum pemphigoid antibody panel demonstrated positive IgG (1:160) and IgA (1:20) basement membrane zone antibodies via monkey esophagus substrate and a positive IgG titer of 1:320 with an epidermal (roof) pattern via human split-skin substrate. ELISA testing revealed elevated IgG BP180 antibodies (41 U/mL, normal <9 U/mL), with normal levels of BP230 (6 U/mL, normal <9 U/mL) and desmoglein 1 and 3 antibodies. Punch biopsy was not performed owing to the absence of active buccal or skin lesions.

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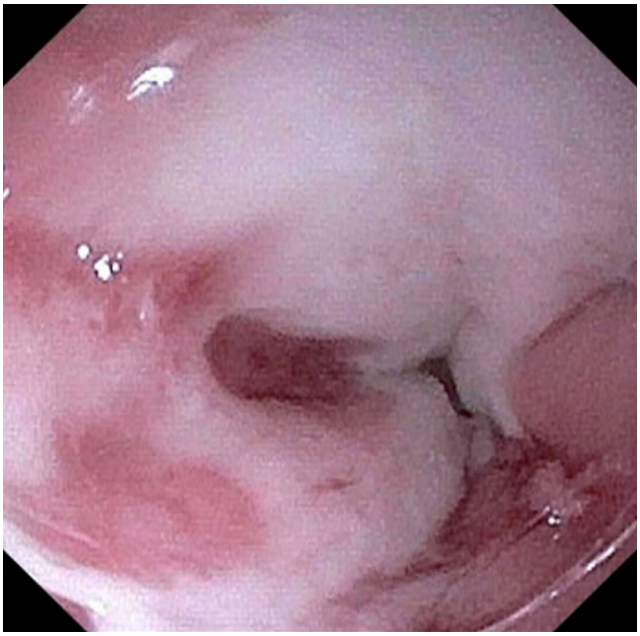

**Figure 1.** *Clinical images from esophagogastroduodenoscopy showing the middle third of the esophagus with ulceration and LA Grade D esophagitis (1 or more mucosal breaks involving at least 75% of the esophageal circumference).*

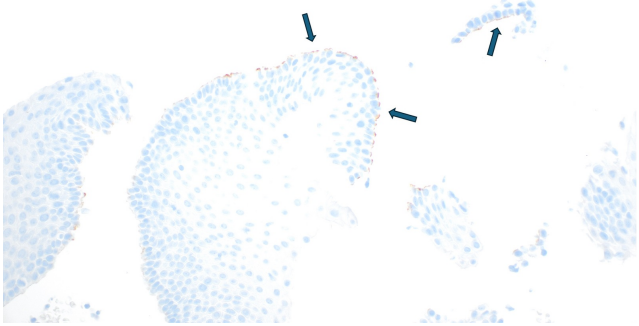

**Figure 2.** *Linear staining along the floor of the basal layer of the subepithelial split, highlighted by arrows (C4d immunohistochemistry, original magnification ×40).*

Upon dermatology follow-up, MMP was suspected. Dermatopathological review of the pre-existing esophageal biopsy revealed focal C4d staining along the basal layer of the subepithelial split, supporting an immunobullous process consistent with MMP (Figure 2). Following diagnosis, the patient continued colchicine 0.6 mg twice daily and initiated dapsone 50 mg daily, reporting notable improvements in pain, swallowing, and weight gain after 5 months. She remains well-controlled on colchicine and dapsone 8 months later and is under close monitoring. She is up to date on cancer screenings, including colonoscopy, Pap smear, and mammogram.

## Case Discussion

This case highlights the diagnostic challenges of MMP, a mucous-membrane–predominant pemphigoid variant that is often difficult to distinguish from other subepidermal blistering disorders, including BP with mucosal involvement, an important differential in this patient.[3] Both BP and MMP are thought to result from autoantibodies targeting basement membrane proteins.[3] Although BP typically presents with cutaneous bullae and less frequently affects mucous membranes, atypical variants with isolated or prominent mucosal disease have been described and may clinically mimic MMP.[8,9] In the present case, the absence of cutaneous lesions, chronicity of mucosal symptoms, and negative BP230 antibody levels supported the diagnosis of MMP. MMP can present with variable cutaneous involvement but primarily affects mucous membranes, and without treatment may lead to mucosal and ocular scarring.[3,4] Esophageal involvement, observed in approximately 2% to 7% of MMP patients, may rarely be the first sign of disease and can result in debilitating stricture and stenosis.[4,10]

Although clinical suspicion may arise based on characteristic features, definitive diagnosis of MMP often requires histopathological confirmation. Cutaneous or oral mucosal biopsies may be unavailable, particularly in predominantly esophageal disease. Although DIF remains the diagnostic gold standard, its reliance on non–formalin-fixed fresh tissue presents logistical challenges, as gastroenterologists performing esophagogastroduodenoscopy may not suspect this rare disease and may be unfamiliar with the requirements for DIF on a pathologic specimen.[1-5] DIF is not routinely performed on esophageal biopsies, which often show inflammation with a broad differential, including eosinophilic, erosive, infective, lichen planus, or pill esophagitis.[11] Determining the etiology of the inflammation and ulceration can therefore be difficult.

C4d IHC on formalin-fixed tissue has emerged as a promising adjunct to DIF in BP and MMP diagnosis, with studies demonstrating comparable sensitivity for detecting linear basement membrane immunoreactant deposition.[1-7] The esophagus, like the oral mucosa, has stratified squamous epithelium and is non-keratinized, similar to regions of the oral mucosa not exposed to mastication.[12] Given C4d's demonstrated diagnostic utility in oral mucosal squamous epithelium, its application to esophageal tissue is reasonable.[1,3,4] The present case suggests that C4d IHC on formalin-fixed mucosal or esophageal biopsies can serve as a practical ancillary diagnostic tool, particularly when fresh tissue is unavailable. A key advantage of this approach is the ability to use existing biopsy specimens, potentially sparing the patient an additional invasive procedure, which is especially relevant in esophageal-predominant disease that requires endoscopy and sedation.

## Conclusion

We describe the use of C4d IHC to retroactively support the diagnosis of MMP in a previously nonspecific formalin-fixed esophageal biopsy, in the absence of active oral or cutaneous lesions. C4d staining on formalin-fixed tissue offers a potential adjunct for diagnosing bullous disorders with mucosal or esophageal involvement, facilitating more efficient diagnosis and management. Although this case illustrates the potential utility of C4d immunostaining in esophageal biopsies, it represents a single case, and the findings should be interpreted with caution. Further studies are needed to validate the diagnostic role of C4d IHC in autoimmune bullous diseases.

### Potential conflicts of interest

The authors declare no conflicts of interest.

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