

# Bullous Pemphigoid: Use of C4d Immunofluorescent Staining in a Case With Repeated Negative Conventional Direct Immunofluorescence Studies

Sarah S. Kassaby, MD,\* Alexander Hicks, BS,† Stuart Leicht, MD,‡ and George A. Youngberg, MD\*

**Abstract:** Direct immunofluorescence (DIF) using frozen section material from a fresh/preserved perilesional biopsy is the gold standard for the immunopathologic diagnosis of bullous pemphigoid (BP). DIF in BP shows linear dermoepidermal junction (DEJ) staining for C3, with or without staining for IgG. In some situations, only a formalin-fixed lesional biopsy is obtained (with no fresh/preserved perilesional biopsy for DIF). In this setting, paraffin section C4d immunohistochemistry has proven to be diagnostically useful, demonstrating linear DEJ positivity for C4d. We present a novel use of C4d staining for the diagnosis of BP, specifically analyzing C4d perilesional frozen section DIF in a case where standard perilesional frozen section DIF for IgG/C3 was available, but was negative. An 80-year-old woman presented with a pruritic bullous lesion on her left upper extremity, clinically thought to represent BP. Lesional histologic findings were typical for BP, but perilesional frozen section DIF staining was negative for IgG and C3. A second set of biopsies processed at a different laboratory yielded the same result. A diagnosis of bullous scabies was considered. Subsequently, perilesional frozen section DIF for C4d was obtained, which showed strong linear DEJ positivity, confirming the diagnosis of BP. DIF for C4d is widely used in transplant pathology, since C4d is persistent in tissue, versus C3. Our case demonstrates that perilesional frozen section DIF staining for C4d may be positive and diagnostic in BP, even when conventional DIF staining for IgG and C3 is negative.

**Key Words:** bullous pemphigoid, C4d, Immunofluorescence, C3, IgG

(*Am J Dermatopathol* 2017;0:1–3)

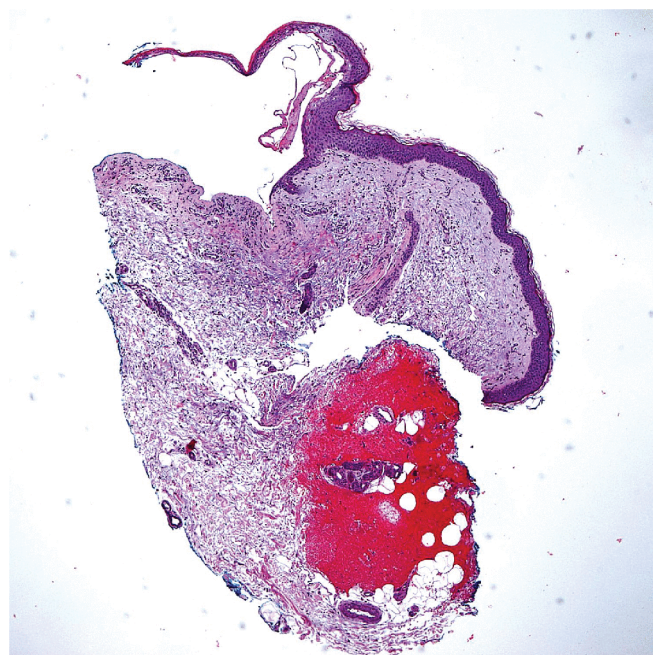
## INTRODUCTION

Bullous pemphigoid (BP) is an autoimmune blistering disorder that affects elderly patients and presents as large tense bullae involving trunk, extremities, and intertriginous

areas. The diagnosis of an autoimmune bullous dermatosis relies greatly on direct immunofluorescence (DIF) examination performed on frozen tissue sections, showing deposits of immunoglobulins and/or C3 on specific cutaneous structures.<sup>1</sup> BP typically demonstrates linear dermoepidermal junction (DEJ) staining for C3 and often IgG, confirming, in context, the clinical and light microscopic diagnosis.

However, in some situations, only a formalin-fixed lesional biopsy is obtained (with no fresh/preserved perilesional biopsy for DIF). In this setting, paraffin section C4d immunohistochemistry has proven to be diagnostically useful, demonstrating linear DEJ positivity for C4d in BP. The validity of this technique has been established by performing paraffin section immunohistochemistry C4d staining in cases of BP where frozen section C3d and/or IgG DIF was known to be positive (along with negative staining in nonimmunobullous biopsies).<sup>1,2</sup>

As far as we are aware, this is the first reported use of C4d DIF staining to confirm a case of BP where conventional DIF for IgG/C3 was obtained but was negative.



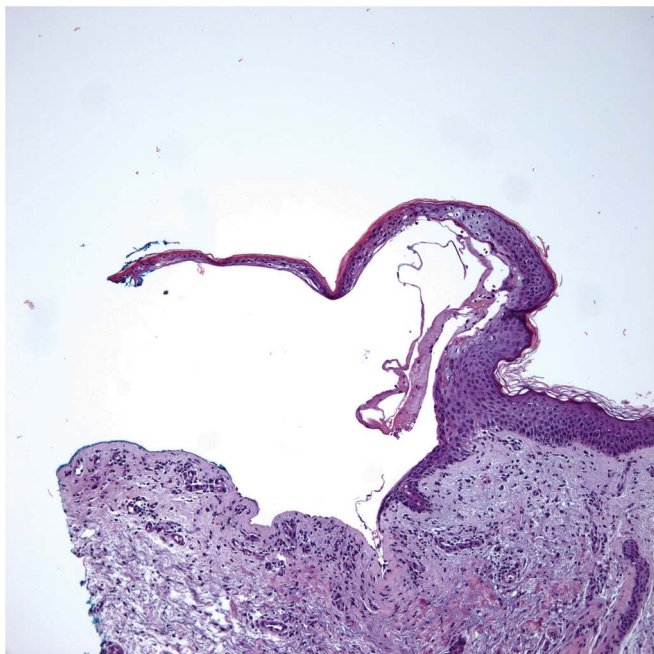
**FIGURE 1.** Low-power view of lesional punch biopsy showing a subepidermal vesicle (hematoxylin and eosin,  $\times 40$ ).

From the \*Department of Pathology, East Tennessee State University, Quillen College of Medicine, Johnson City, TN; †James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN; and ‡Department of Medicine, East Tennessee State University, Quillen College of Medicine, Johnson City, TN.

The authors declare no conflicts of interest.

Reprints: Sarah S. Kassaby, MD, Department of Pathology, East Tennessee State University, PO Box: 70568, Johnson City, TN 37614 (e-mail: kassaby@etsu.edu).

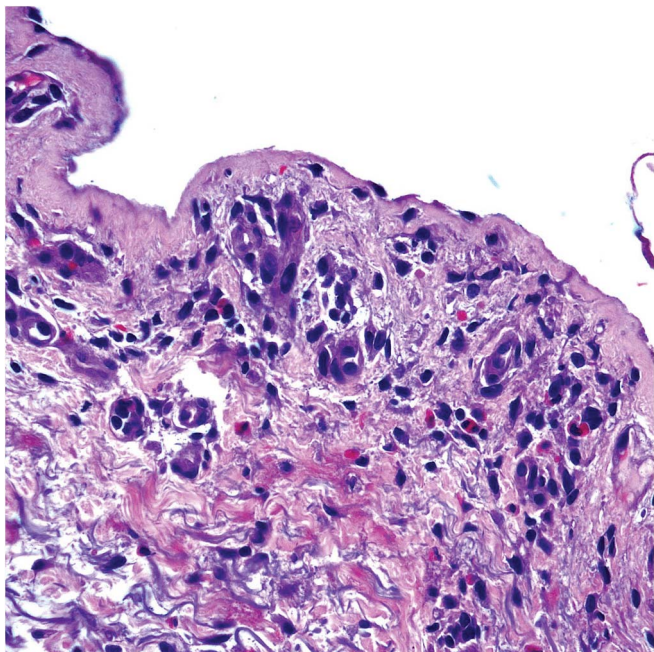
Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.



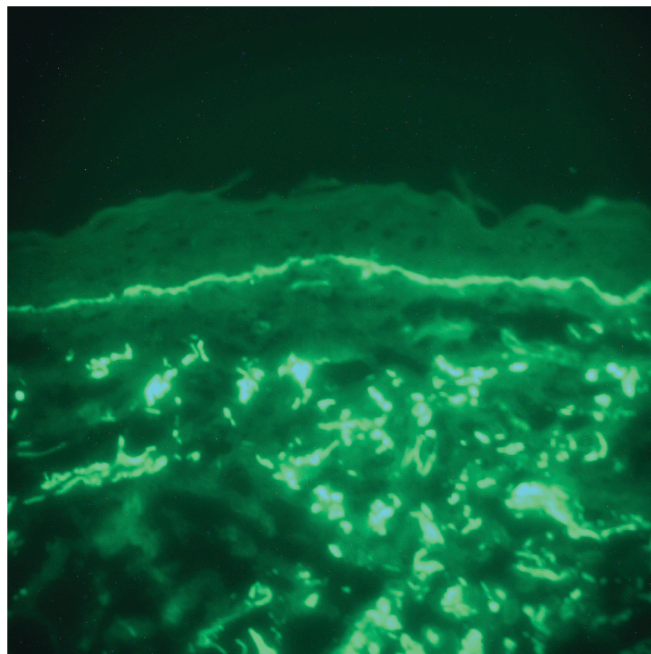
**FIGURE 2.** Subepidermal vesicle with eosinophil-predominant inflammation (hematoxylin and eosin,  $\times 200$ ).

## MATERIALS AND METHODS

An 80-year-old woman presented with a pruritic bullous lesion on her left upper extremity, clinically thought to represent BP, although a differential diagnosis of bullous scabies was also considered. A lesional punch biopsy for



**FIGURE 3.** Upper dermis showing perivascular lymphohistiocytic infiltrate accompanied by conspicuous eosinophils (hematoxylin and eosin,  $\times 400$ ).



**FIGURE 4.** Perilesional frozen section DIF for C4d, showing strong linear DEJ positivity ( $\times 400$ ).

histologic evaluation and a perilesional punch biopsy for DIF were both obtained.

## RESULTS

Microscopically, the lesional punch biopsy showed a subepidermal vesicle with eosinophil-predominant inflammation, typical for BP (Figs. 1–3). Multiple levels were examined, with no findings of bullous scabies. However, the perilesional punch biopsy showed no direct immunofluorescent staining for IgG, IgM, IgA, or C3. Biopsies previously evaluated by an independent laboratory had also demonstrated histologic features compatible with BP, associated with negative DIF.

After the 2 negative conventional DIF studies, perilesional frozen section DIF for C4d, performed in our laboratory, showed strong linear DEJ positivity, helping to confirm the diagnosis of BP (Fig. 4). Subsequently, serum testing for BP was also obtained, performed by Quest Diagnostics. BP180 testing was negative (less than 5 U/mL), but BP230 (IgG) testing was positive (17 U/mL vs. an upper reference range of  $<9$  U/mL). Indirect immunofluorescence testing and salt-split skin DIF were not obtained in this case.

## DISCUSSION

DIF for C4d is currently widely used in transplant pathology, since C4d is stable and persistent in tissue, versus C3 (the C4d complement fragment is known to bind covalently to endothelial cells).<sup>3</sup> Biopsy diagnosis of antibody-mediated renal allograft rejection has been greatly



enhanced by the inclusion of C4d staining (DIF or immunohistochemistry) into the Banff Classification.

The patient in our case was strongly suspected clinically to have BP and lesional biopsies were histologically typical for BP. However, DIF was repeatedly negative. We hypothesized that C4d staining might be positive, although C3 DIF staining was negative, because of the stability of C4d and the possibility of persistent C4d-tissue binding in the basement membrane zone region. Since our renal biopsy service already had a DIF C4d stain set up, we stained a frozen section from the perilesional skin biopsy in this case for DIF C4d, and demonstrated strong linear DEJ positivity, helping to confirm the diagnosis of BP.

C4d staining in the context of immunobullous disease functions as a surrogate for C3 staining (in the presence of classical or lectin pathway activation). Therefore, positive linear DEJ staining might occur in several other autoimmune subepidermal blistering dermatoses, including various forms of cicatricial pemphigoid, anti-p200 pemphigoid, pemphigoid gestationis, epidermolysis bullosa acquisita (EBA), bullous lupus, lichen planopilaris, and various forms of linear IgA dermatosis. The primary clinical differential in this patient was BP, with a secondary consideration of bullous scabies. The patient had no mucosal involvement, no scarring, no clinical features of lichen planus or lupus, and was not pregnant. IgA staining was negative.

Perhaps the most challenging differential was EBA. Clinically, the dermatologist favored BP, but EBA cannot always be excluded on a clinical basis. EBA is usually noninflammatory, whereas this biopsy showed prominent eosinophilic inflammation. However, occasional cases of EBA do show eosinophilic inflammation. DIF on salt-split skin would have helped to resolve any lingering consideration of EBA, but this test was not performed. It is our opinion, though, that C4d staining in this case showed an n-serration pattern consistent with pemphigoid, while EBA or bullous lupus would have demonstrated a u-serration pattern.<sup>4-6</sup>

Regarding bullous scabies, no fecal material or organisms were detected on multiple section levels, but this does not totally exclude a diagnosis of scabies. The linear C4d DEJ staining militates against a bullous scabies diagnosis, although a few cases of scabies with a DIF-positive BP-like eruption have been reported.<sup>7-9</sup> On follow-up, our patient responded to antipemphigoid treatment and no evidence of scabies has emerged.

We are not certain why the conventional DIF in this case was negative. The patient was not known to be immunosuppressed and was not on steroid therapy or any other anti-BP therapy at the time of our biopsy. According to the dermatologist, this patient had mild disease (limited to trunk and 1 arm, and with no more than 3 lesions present at any one time). It has been reported that DIF in BP can be weak or negative in cases with localized (milder) disease.<sup>10,11</sup>

As far as we are aware, this is the first reported use of C4d DIF staining to confirm a case of BP where the conventional DIF for IgG/C3 was negative. Our case clearly demonstrates that perilesional frozen section DIF staining for C4d may be positive and diagnostically useful in BP, even when the conventional DIF staining for IgG and C3 is negative. Further studies will be required to confirm the potential utility (and sensitivity, specificity, and predictive values) of C4d staining to support a diagnosis of likely cases of BP in which standard DIF is negative.

## REFERENCES

1. Villani AP, Chouvet B, Kanitakis J. Application of C4d immunohistochemistry on routinely processed tissue sections for the diagnosis of autoimmune bullous dermatoses. *Am J Dermatopathol*. 2016;38:186-188.
2. Chandler W, Zone J, Florell S. C4d immunohistochemical stain is a sensitive method to confirm immunoreactant deposition in formalin-fixed paraffin-embedded tissue in bullous pemphigoid. *J Cutan Pathol*. 2009;36:655-659.
3. González-Molina M, Ruiz-Esteban P, Caballero A, et al. Immune response and histology of humoral rejection in kidney transplantation. *Nefrologia*. 2016;36:354-367.
4. High WA. Blistering diseases. In: Elston DM, Furrer T, eds. *Dermatopathology*. 2nd ed. Philadelphia, PA: Saunders Ltd; 2014:168-169.
5. Mehren CR, Gniadecki R. Epidermolysis bullosa acquisita: current diagnosis and therapy. *Dermatol Rep*. 2011;3:e38.
6. Vodegel RM, Jonkman MF, Pas HH, et al. U-serrated immunodeposition pattern differentiates type VII collagen targeting bullous diseases from other subepidermal bullous autoimmune diseases. *Br J Dermatol*. 2004;151:112-118.
7. Balighi K, Robati RM, Hejazi N. A dilemma: bullous-pemphigoid-like eruption in scabies or scabies-induced bullous pemphigoid. *Dermatol Online J*. 2006;12:13.
8. Slawsky LD, Maroon M, Tyler WB, et al. Association of scabies with a bullous pemphigoid-like eruption. *J Am Acad Dermatol*. 1996;34:878-879.
9. Bhawan J, Milstone E, Malhotra R, et al. Scabies presenting as bullous pemphigoid-like eruption. *J Am Acad Dermatol*. 1991;24:179-181.
10. Person JR, Rogers RS III, Perry HO. Localized pemphigoid. *Br J Dermatol*. 1976;95:531-534.
11. Weigand DA, Clements MK. Direct immunofluorescence in bullous pemphigoid: effects of extent and location of lesions. *J Am Acad Dermatol*. 1989;20:437-440.