



HAL
open science

Application of C4d Immunohistochemistry on Routinely Processed Tissue Sections for the Diagnosis of Autoimmune Bullous Dermatoses

Axel P Villani, Brigitte Chouvet, Jean Kanitakis

► **To cite this version:**

Axel P Villani, Brigitte Chouvet, Jean Kanitakis. Application of C4d Immunohistochemistry on Routinely Processed Tissue Sections for the Diagnosis of Autoimmune Bullous Dermatoses. *American Journal of Dermatopathology*, 2016. hal-04953255

HAL Id: hal-04953255

<https://hal.science/hal-04953255v1>

Submitted on 18 Feb 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Application of C4d Immunohistochemistry on Routinely Processed Tissue Sections for the Diagnosis of Autoimmune Bullous Dermatoses

Axel P. Villani, MD,*† Brigitte Chouvet, MD,*† and Jean Kanitakis, MD*†

Abstract: The diagnosis of autoimmune bullous dermatoses relies greatly on direct immunofluorescence (DIF) examination performed on frozen tissue sections, showing deposits of immunoglobulins and/or C3 on specific cutaneous structures. However, frozen material is not always available for DIF; therefore, alternative techniques are needed in the diagnostic procedure. We therefore tested the usefulness of C4d immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections for the diagnosis of bullous pemphigoid (BP) and pemphigus (P). A retrospective immunohistochemical study was performed on biopsies of BP (n: 29) and P (n: 22, including 19 *Pemphigus vulgaris* and 3 paraneoplastic), submitted for routine histological examination and compared with DIF on the corresponding frozen sections. Twenty-five BP biopsies (86.2%) showed C4d deposits seen as a linear labeling along the dermal-epidermal junction and on the membrane of basal keratinocytes. Seventeen P biopsies (77.2%) showed C4d deposits in a classical “intercellular” pattern, predominating on the lower epidermal layers. The sensitivity, specificity, positive predictive value, and negative predictive value reached 86%, 98%, 96%, and 92% in BP, respectively and 77%, 98%, 94%, and 92% in P, respectively. Furthermore, in the cases where serological tests were available, the sensitivity of C4d detection was higher than that of enzyme-linked immunosorbent assay/indirect immunofluorescence in both BP (87% vs. 67%) and P (82% vs. 54.5%). We conclude that DIF on frozen sections still remains the gold standard for the immunopathological diagnosis of BP and P; however, in the absence of frozen material, C4d immunohistochemistry performed on routinely processed biopsy material can be of considerable help in confirming the diagnosis.

Key Words: bullous pemphigoid, pemphigus, formalin-fixed paraffin-embedded tissue sections, C4d immunohistochemistry, direct immunofluorescence, indirect immunofluorescence, ELISA

(*Am J Dermatopathol* 2016;38:186–188)

From the Departments of *Dermatology, and †Pathology, Edouard-Herriot Hospital, Lyon University, Lyon, France.

The authors declare no conflicts of interest.

All authors have sufficiently participated in the submitted work to deserve authorship; all have had access to the pathological material and have approved the submitted article. A. P. Villani endorses the scientific responsibility of the work reported herein.

Reprints: Axel P. Villani, MD, Service de Dermatologie, Hôpital Edouard Herriot, 69437 Lyon Cedex 03, France (e-mail: axel.villani@chu-lyon.fr). Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

INTRODUCTION

Bullous pemphigoid (BP) and pemphigus (P) account for 2 of the most common autoimmune bullous dermatoses (AIBD). Their diagnosis can be suggested clinically but must be confirmed by a pathological study, including examination of routinely processed [formalin-fixed, paraffin-embedded (FFPE)] sections of a skin biopsy taken (ideally) from the edge of a recent bullous lesion, and direct immunofluorescence (DIF) testing of a snap-frozen (or fixed in Michel’s medium before freezing) skin biopsy taken from peribullous skin. DIF typically shows linear IgG and/or C3 deposits along the dermal-epidermal junction (DEJ) in BP, and IgG and C3 deposits on the surface of epidermal keratinocytes (the so-called “intercellular” pattern) in P. Although DIF is essential in the diagnosis of both BP and P, suitable skin specimens for DIF (ie, snap-frozen or fixed in Michel’s medium) are not always available to the pathologist, either because an AIBD was not suspected by the submitting physician (several clinically atypical presentations of P and BP exist), or because of the difficulty to handle and forward frozen skin samples suitable for DIF examination to the laboratory. When antigen retrieval techniques became available, attempts were made to perform DIF on FFPE skin biopsies using these techniques (enzymatic treatment and microwave heating in citrate or urea buffer). These early studies achieved detection of immunoglobulins and/or C3 in a characteristic pattern in 50%–60% of BP and P cases.^{1,2} More recently, commercially available mouse monoclonal and rabbit polyclonal antibodies have been produced that are able to reliably detect human C3d and C4d, stable component of classic complement activation, on FFPE biopsies using immunoenzymatic techniques. The usefulness of immunohistochemical detection (IHC) of C3d and C4d in the diagnosis of inflammatory dermatoses, including AIBD, has been addressed in some studies^{3–6}; these included a limited number of biopsies or studied a variety of inflammatory dermatoses without targeting specifically AIBD, and their results were sometimes contradictory.⁶ The purpose of this study was to further assess the usefulness of immunoperoxidase detection of C4d in FFPE skin specimens in the diagnosis of BP and P.

MATERIALS AND METHODS

A retrospective immunohistochemical study was performed on all cases of BP (n: 29) and P (n: 22, including 19 *Pemphigus vulgaris* and 3 paraneoplastic P cases) that had been diagnosed over the past 6 years in our Dermatopathology

laboratory, for which both FFPE and fresh-frozen diagnostic skin biopsy had been submitted concomitantly. The diagnosis had been confirmed in each case on the basis of a characteristic aspect on routinely stained skin sections (subepidermal and intraepidermal blistering in BP and P, respectively) and by positive DIF test on the corresponding frozen skin specimen, showing deposits of IgG and/or C3 at the DEJ in BP, and on the surface of epidermal keratinocytes in P. Skin sections of the corresponding FFPE biopsies of all these cases were labeled immunohistochemically in a Ventana immunohistochemistry automate, using a rabbit polyclonal antiserum to C4d (Biomedica Gruppe, Austria) (applied at a dilution of 1:30 for 30 minutes at room temperature after antigen retrieval, according to the instructions provided by the manufacturer). Diaminobenzidine was used as chromogen, and Harris hematoxylin as counterstain. To assess the specificity of the immunolabeling, we performed in parallel C4d immunostaining of FFPE skin biopsies of potential histologic mimics of BP and P, including cases of subacute/vesicular dermatitis (n: 12), herpetic infection blisters (n: 6), bullous drug eruptions (n: 5), dermatitis herpetiformis (n: 5), nodular prurigo (n: 7), Grover disease (n: 4), and Hailey–Hailey disease (n: 5). Negative controls were performed by omitting the first-layer antibody. The results of serological tests [enzyme-linked immunosorbent assay (ELISA) and/or indirect immunofluorescence (IIF)] when available were recorded and compared with the results of C4d detection.

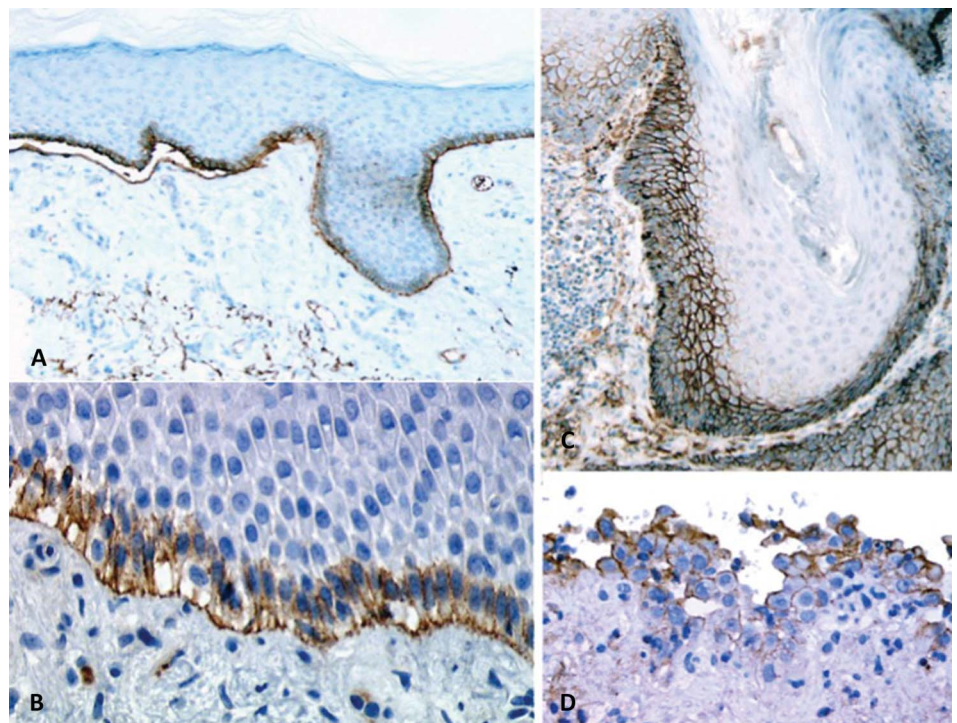
RESULTS

Of 29 BP biopsies, 25 (86.2%) were C4d+. The C4d deposits were visualized as a brown linear labeling along the

DEJ (found at the level of the blister and in noncleaved skin) and as labeling of the membrane of basal keratinocytes (Figs. 1A, B). Whenever the biopsies contained bullous lesions, C4d immunolabeling was as a rule found both at the roof and the floor of the blister. Eight biopsies of BP included nonbullous lesions; 6 of them were C4d+. All the C4d+ BP cases had linear C3 deposits on the DEJ by DIF on frozen specimens. Three of the 4 BP biopsies that were C4d– had linear C3 and IgG deposits along the DEJ by DIF on frozen specimens (the fourth one had IgG, but no C3, deposits). Regarding P, 17 of 22 biopsies (77.3%) were C4d+. The staining of C4d had a similar aspect to the one seen by DIF on frozen skin specimens, that is, it was visualized as brown deposits on the surface of epidermal keratinocytes, predominating on the lower epidermal layers (“intercellular” pattern) (Fig. 1C). C4d deposits were also found on the surface of acantholytic keratinocytes present in the blisters (Fig. 1D). Of the 44 control biopsy specimens, only 1 (2.3%) showed C4d labeling that was considered specific; this was a biopsy of dermatitis herpetiformis that showed granular deposits of IgA on the tips of dermal papillae. Of note, immunostaining for C4d was regularly found on dermal (presumably elastic) fibers and within apoptotic/necrotic keratinocytes, seen in cases of H and bullous drug eruptions, of unknown specificity. Based on these results, the sensitivity, specificity, positive predictive value, and negative predictive value were evaluated at 86%, 98%, 96%, and 94% in BP, respectively and 77%, 98%, 94%, and 92% in P, respectively.

Serological data (ELISA and/or IIF) were available for 15 of the 29 cases of BP and 11 of the 22 cases of P included in this study. Analysis of these cases showed that the rate of detection of C4d was higher than that of ELISA/IIF. In BP,

FIGURE 1. A, C4d immunostaining in a bullous lesion of BP shows linear deposits at the DEJ associated with deposits on the surface of basal epidermal keratinocytes. C4d labeling is present both at the level of dermal-epidermal separation (left) and on noncleaved skin (right) (original magnification $\times 100$). B, BP. Higher magnification shows C4d deposits on the DEJ and on the surface of epidermal keratinocytes (original magnification $\times 250$). C, Pemphigus. IHC for C4d shows “intercellular” deposits of C4d within the lower epidermis (original magnification $\times 100$). D, At higher magnification, C4d deposits are seen on the surface of acantholytic cells lining the intraepidermal bulla (original magnification $\times 250$).



the positivity rate reached 87% for C4d versus 67% for ELISA/IIF. In P, the rate of detection was 82% for C4d versus 54.5% for ELISA/IIF.

DISCUSSION

IHC for C3d and C4d on FFPE tissue sections has been tested in a small number of studies as an alternative to DIF for the diagnosis of autoimmune dermatoses. Pfaltz et al³ found that 31 of 32 cases of BP (97%) showed linear deposits of C3d at the DEJ. Magro and Dyrsen⁴ studied 17 cases of BP with C3d and C4d IHC and found invariable concordance between C3d IHC and classical DIF but (somewhat unexpectedly) did not detect C4d positivity in BP cases. This contrasts with the results of our study that showed 86% of BP cases to be C4d+. This discrepancy could be because of differences in specimen handling (type of fixative and fixation duration) and in immunostaining protocols. Our results are in agreement with those of Chandler et al,⁶ who found linear C4d positivity on the DEJ in 7 of 9 of BP cases. Regarding P, Magro and Dyrsen found that 82% of cases had intercellular C3d and C4d deposits, roughly mirroring the intercellular pattern for IgG and complement seen in DIF. This result is very similar to our findings, showing C4d positivity in 77.3% of P cases. In our study, the sensitivity of C4d detection by IHC on FFPE for the diagnosis of BP and P (86% and 77%, respectively) was found higher than that of DIF performed after antigen retrieval in FFPE (ca. 55%)² but remained lower than that of traditional DIF on frozen skin specimens. However, the specificity of this technique for both BP and P seems high (97% and 100%, respectively). Our results show that, similarly to DIF, C4d immunohistochemistry on FFPE tissue sections is more sensitive than serological examinations (ELISA and IIF)

for the diagnosis of BP and P, as serologically negative BP and P patients have C4d+ skin biopsies in 27% and 55% of cases, respectively.

In conclusion, DIF on frozen skin specimens still remains the gold standard, first-choice adjunct to routine microscopic study in the diagnosis of BP and P by virtue of its high sensitivity. However, when frozen material is not available, C4d IHC performed on FFPE material submitted for routine microscopic study can confirm the diagnosis of BP and P in the majority of cases, thereby obviating the necessity to obtain an additional biopsy and accelerating the diagnostic procedure. Furthermore, this technique can be used for retrospective studies as FFPE material remains usually available for several years.

REFERENCES

1. D'Ambra-Cabry K, Deng DH, Flynn KL, et al. Antigen retrieval in immunofluorescent testing of bullous pemphigoid. *Am J Dermatopathol*. 1995; 17:560–563.
2. Al-Rifai I, Kanitakis J, Faure M, et al. Immunofluorescence diagnosis of bullous dermatoses on formalin-fixed tissue sections after antigen retrieval. *Am J Dermatopathol*. 1997;19:103–105.
3. Pfaltz K, Mertz K, Rose C, et al. C3d immunohistochemistry on formalin-fixed tissue is a valuable tool in the diagnosis of bullous pemphigoid of the skin. *J Cutan Pathol*. 2010;37:654–658.
4. Magro CM, Dyrsen ME. The use of C3d and C4d immunohistochemistry on formalin-fixed tissue as a diagnostic adjunct in the assessment of inflammatory skin disease. *J Am Acad Dermatol*. 2008;59:822–833.
5. Kwon EJ, Ntiamoah P, Shulman KJ. The utility of C4d immunohistochemistry on formalin-fixed paraffin-embedded tissue in the distinction of polymorphic eruption of pregnancy from pemphigoid gestationis. *Am J Dermatopathol*. 2013;35:787–791.
6. 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.