Check for updates

JEADV Clinical Practice

SHORT REPORT OPEN ACCESS

Buccal Mucosa as Substrate for Direct Immunofluorescence Should Be Included Early in the Diagnostic Work Up of Ocular Mucous Membrane Pemphigoid

Andrea Gabusi[1] | Michelangelo La Placa[2,3] | Davide Bartolomeo Gissi[1] | Federica Filippi[2,3] | Camilla Loi[2,3] | Cosimo Misciali[2,3] | Federico Bardazzi[2,3]

[1]Section of Oral Sciences, Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy | [2]Department of Experimental Diagnostic and Specialty Medicine, Division of Dermatology, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy | [3]Division of Dermatology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

Correspondence: Michelangelo La Placa (michelangelo.laplaca@unibo.it)

Received: 6 December 2024 | **Revised:** 27 October 2025 | **Accepted:** 13 November 2025

Funding: The authors received no specific funding for this work.

Keywords: direct immunofluorescence | ocular pemphigoid | oral mucosa biopsy

ABSTRACT

Background: In ocular mucous membrane pemphigoid (MMP) direct immunofluorescence (DIF) represents the gold standard for definitive diagnosis. However, up to 50% of ocular MMP cases do not meet the immunopathological criteria. In addition, the risk of exacerbating conjunctival cicatrization makes ocular biopsy technically difficult to obtain. Despite current guidelines suggesting that biopsies could occasionally be taken from non-ocular sites if the conjunctiva is inflamed, studies describing the use of buccal mucosa as a substrate for DIF are scarce.

Objectives: The aim of the present study was to describe the utility of DIF in buccal samples from patients with suspected ocular MMP and contraindication for conjunctival biopsy.

Methods: Medical records of patients with suspected ocular MMP were retrospectively reviewed. Inclusion criteria were (1) clinical diagnosis of ocular pemphigoid; (2) contraindication for conjunctival biopsy; (3) DIF performed on buccal mucosa; (4) ELISA tests for BP180 and BP230 at presentation. DIF results were compared with ELISA tests and clinical data.

Results: Nine patients met the inclusion criteria. DIF positivity was seen in four out of nine (44%) patients, confirming the diagnosis of ocular MMP. Three out of four (75%) patients with positive DIF were off treatment. By contrast, five out of five (100%) with non-specific/negative DIF results were on prolonged (> 6 months) immunosuppressive therapy. Only one out of nine (11%) showed circulating anti-BP180 Abs and anti-BP230 Abs exceeding the cut-off of 20 U/mL (44 and 88 U/mL, respectively).

Conclusions: Buccal DIF is a very attractive procedure for confirming diagnosis in suspected ocular MMP patients when conjunctival biopsy is unavailable and appears superior to BP180/BP230 ELISA. The higher prevalence of positive results in patients off-therapy suggests that the diagnostic yield may improve if buccal DIF is performed before treatment.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *JEADV Clinical Practice* published by John Wiley & Sons Ltd on behalf of European Academy of Dermatology and Venereology.

1 | Background

Mucous membrane pemphigoid (MMP) is a rare autoimmune subepithelial blistering disease. The pathogenesis of MMP has not been completely elucidated, but involvement of an inflammatory reaction mediated by antibodies against autoantigens, in association with complement (C3) activation and immune cell recruitment, has been reported. At least six autoantigens have been identified: bullous pemphigoid (BP) antigen (180 kDa; BP180), BP antigen (230 kDa; BP230), α6β4 integrin, laminin 332 and type VII collagen (COLVII) [1].

Ocular involvement occurs in 60%–70% MMP cases, and it is characterized by chronic conjunctivitis and scarring, ultimately leading to blindness [2].

The diagnosis of ocular MMP is often challenging. Although ocular direct immunofluorescence (DIF) represents the gold standard for definitive diagnosis, up to 50% of ocular MMP cases do not meet the immunopathological criteria [3]. In such cases, diagnostic confirmation cannot be obtained, despite clinical phenotype, disease severity and disease course being identical to ocular MMP cases with a positive immunopathology [4].

To further complicate the situation, obtaining a conjunctival biopsy for immunopathological analysis can also be challenging. Patients can easily refuse to undergo a surgical procedure that is not free from risk. In addition, according to a recent survey, up to 28% of ophthalmologists do not perform ocular biopsy in suspected cases of MMP due to limited access to specialized laboratories and the risk of exacerbating cicatrization [5].

Cases of suspected ocular MMP where conjunctival DIF is negative or not available (either because of patient refusal or clinical contraindications) represent a delicate clinical problem. Indeed, given the risks of progressive clinical worsening and cicatrization, some physicians may opt to treat empirically based on clinical presentation [6]. The rationale for this approach is aimed at preventing visual loss by promptly treating progressive cicatrizing conjunctivitis. However, as a drawback, some cases not related to MMP may undergo systemic immunosuppression. Hence, strategies to improve diagnostic confirmation in these particular settings are highly warranted.

Current guidelines suggest that biopsies could occasionally be taken from non-ocular sites if the conjunctiva is inflamed [3]. Oral mucosa is easily accessible and does not undergo scarring. Nevertheless, studies on buccal mucosa are scarce [3, 7], recommendations for oral DIF are lacking, and oral DIF is not frequently performed [5].

2 | Objectives

The aim of the present study was to describe the utility of DIF in buccal samples for diagnostic confirmation in patients with suspected ocular MMP, particularly in cases where conjunctival biopsy is unavailable.

3 | Methods

Following ethical committee approval (Code OBLI01), we retrospectively reviewed the medical records of all patients treated for ocular pemphigoid that were followed at the Division of Dermatology Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, from 2017 to 2024, and underwent oral DIF due to the unavailability of conjunctival biopsy. The aim was to describe trends in oral DIF samples and investigate positivity rates in comparison with ELISA tests and clinical data.

Inclusion criteria were as follows: (1) strong clinical suspicion of localized ocular pemphigoid after ophthalmological examination and the exclusion of other causes of chronic conjunctivitis. In particular, a high level of clinical suspicion for OCP was defined as the presence of a typical scarring pattern or conjunctival inflammation and the absence of an alternative aetiology of cicatrizing conjunctivitis. (2) Unavailability of conjunctival biopsy because it was judged at elevated risk of cicatrization during the diagnostic work up. (3) DIF performed on buccal mucosa. (4) Results of ELISA tests for BP180 and BP230 at presentation.

Buccal DIF biopsies were performed by an oral medicine specialist (A. G.) from non-affected oral mucosa (Figure 1A–C). Fresh frozen samples were tested for IgA, IgM, IgG and C3 deposits. The analysis was carried out by a pathologist experienced in blistering diseases (C. M.). Samples exhibiting linear

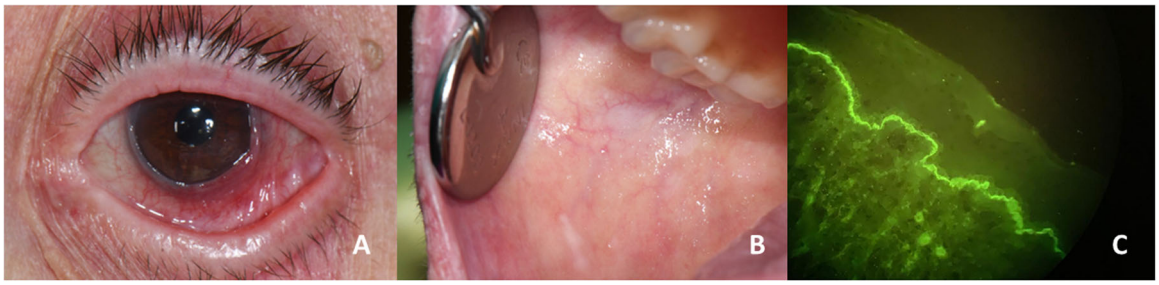

FIGURE 1 | (A) An example of a patient with a clinical diagnosis of mucous membrane pemphigoid (MMP). Signs of conjunctival fibrosis, cicatrization and symblepharon can be noted. Ocular biopsy was judged too risky. (B) Unaffected buccal mucosa can be sampled as a substrate for oral DIF. (C) An example of positive oral DIF from the buccal mucosa of a patient with a clinical diagnosis of MMP and judged contraindicated for ocular biopsy. Linear deposits of IgG along the basal membrane zone can be noted.

27686566, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jvc2.70238 by Area Sistemi Dipart & Doc, Wiley Online Library on [26/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

deposits of IgG, C3 and/or IgA were considered 'positive' and confirmatory of the diagnosis of MMP. All ELISA kits used for investigating circulating anti-Abs against BP180/BP230 were manufactured by Euroimmun Medizinische Labordiagnostika AG, Lubeck (Cut-off 20 U/mL). The following variables were also retrieved: age, sex, disease activity at the time of buccal biopsy, presence/absence of symblepharon, and on/off treatment regimen at the time of buccal biopsy.

More in detail, disease activity at the time buccal DIF was defined as 'stable disease' if, according to clinical records, conjunctival inflammation was controlled, areas of conjunctival cicatrization had not extended, and the clinical situation was reported as 'without evidence of progression' with respect to the latest visit preceding buccal DIF. By contrast, in the presence of persistent uncontrolled conjunctival inflammation, extension or worsening of cicatrization, the term 'active disease' was adopted.

Given the risk of progression of ocular complications, follow-up visits were on average scheduled with a 3–4-month interval.

4 | Results

Nine patients met the inclusion criteria (six females, three males, mean age 74 ± 11, 29 years). At the time buccal biopsy for DIF was sampled, symblepharon was present in four out of nine patients (44%), three out of nine patients were off therapy (buccal biopsy was performed before the onset of systemic immunosuppression), whereas the remaining six patients were under prolonged (> 6 months) immunosuppressive therapy. In particular, two out of nine patients were under cyclosporine eyedrops, one out of nine patients was under tacrolimus eyedrops, five out of nine patients were under prednisone minimal therapy (5 mg daily) and Dapsone (100 mg daily), whereas one out of nine patients was taking mycophenolate mofetil (2 g daily).

Regarding disease activity at the time of buccal biopsy, four out of nine patients showed signs of active disease (clinical signs of uncontrolled conjunctival inflammation or scarring extension that had worsened with respect to the visit preceding buccal biopsy), whereas the remaining five out of nine patients underwent buccal DIF when disease activity was controlled and did not exhibit worsening or progression (stable disease).

Only one out of nine (11%) showed circulating anti-BP180 Abs and anti-BP230 Abs exceeding the manufacturer's cut-off of 20 U/mL (44 and 88 U/mL, respectively).

DIF positivity was seen in four out of nine (44%) patients, confirming the diagnosis of ocular MMP. In two out of nine patients, unspecific results (fluorescent bodies of unknown significance) were noted, whereas completely negative results emerged in three out of nine (33%).

Three out of four (75%) patients with positive DIF were off treatment. By contrast, five out of five (100%) with non-specific/negative DIF results were on prolonged (> 6 months) immunosuppressive therapy. In terms of disease activity, three out of four (75%) patients with positive DIF were classified as having 'active' disease at the time of buccal biopsy, whereas four out of five (80%) patients with non-specific/negative DIF results were in 'stable disease'. Results are summarized in Table 1.

5 | Discussion

Within the limits of the small studied population, our results suggest that in patients with suspected ocular pemphigoid and negative/unavailable conjunctival DIF for excessive inflammation or high risk of cicatrization, the use of buccal mucosa as a substrate for DIF is an attractive strategy to obtain diagnostic confirmation. In agreement with previous reports by Lopez et al. [8] and Jakubowska et al. [7], it emerged that the rate of positive oral DIF is only slightly inferior to the reported rates of conjunctival biopsy, but with considerably less risk.

Indeed, we found a positive DIF in four out of nine (44%) patients, which is similar to the rate of positive buccal DIF at first biopsy found by Lopez et al. (14/41, 34%). By contrast, in the same study, the ocular biopsy yielded a positive DIF rate of 66% but only 18 out of 44 accepted the risk of the conjunctival biopsy.

Only three out of nine patients underwent buccal DIF before the onset of immunosuppression. This data suggests that buccal biopsy for diagnostic confirmation is often considered late. This finding is not surprising, as in clinical practice, it is not uncommon in cases of negative or unavailable conjunctival DIF to initiate immunosuppression, regardless of definitive diagnostic confirmation, to prevent the rapid progression of ocular complications [8].

Curiously, in the present study, three out of four (75%) patients with positive DIF were off treatment. By contrast, more than 50% (six out of nine) of the MMP patients were under prolonged (> 6 months) pharmacological treatment at the time oral DIF was scheduled, of which two out of six patients showed unspecific fluorescence bodies and three out of six were DIF-negative. Collectively, five out of five (100%) with non-specific/negative DIF results were on prolonged (> 6 months) immunosuppressive therapy.

Moreover, in five out of nine patients, buccal biopsy was sampled during a controlled and stable phase of disease activity. Curiously, four out of five (80%) patients sampled in a 'stable' phase of disease activity showed non-specific/negative DIF results.

This has raised the suspicion that in patients experiencing a controlled phase of the disease and under prolonged systemic therapy, the likelihood of detecting IgG deposits in buccal mucosa may be altered. Indeed, systemic treatment, through the reduction of circulating antibodies and the inhibition of local inflammation, may alter results, partly explaining some of the reported unspecific/negative cases [9, 10]. Since only three out of nine patients performed oral DIF off-therapy and during the active phase of the disease, and all three (100%) had positive buccal DIF, in the future, it would be interesting to investigate whether buccal DIF, if performed in early active phases and

27686566, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jvc2.70238 by Area Sistemi Dipart & Doc, Wiley Online Library on [26/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 | Clinical characteristics of patients with a clinical diagnosis of ocular MMP who performed oral DIF.

Patient	Sex	Age at biopsy	Ocular sequelae	Therapy at oral biopsy (duration months)	Disease activity at oral biopsy	Oral DIF	BP180 ELISA (U/mL)	BP230 ELISA (U/mL)
1	M	84	Absent	No therapy	Active disease	Linear IgG/C3 deposits	4	< 2
2	F	74	Symblepharon	CYC eyedrops, CS 5 mg DP 100 mg/day (> 6 months)	Active disease	Unspecific fluorescent bodies	44	83
3	M	86	Absent	No therapy	Active disease	Linear IgG/C3 deposits	< 2	15
4	F	76	Symblepharon	CYC eyedrops CS 5 mg/daily DP 100 mg (> 6 months)	Stable disease	Unspecific fluorescent bodies	< 2	3
5	M	79	Symblepharon	No therapy	Active disease	Linear IgA	< 2	< 2
6	F	53	Absent	TCR eyedrops, Prednisone 5 mg DP 100 mg (> 6 months)	Stable disease	Negative	< 2	< 2
7	F	66	Symblepharon	CS 5 mg Dapsone 100 mg (> 6 months)	Stable disease	Negative	3	2
8	F	85	Absent	PR 5 mg DP 100 mg (> 6 months)	Stable disease	Linear C3 deposits	< 2	< 2
9	F	63	Absent	MPM 2 g (> 6 months)	Stable disease	Negative	19	< 2

Abbreviations: CS, prednisone; CYC, cyclosporine eyedrops; DP, dapsone; MPM, mycophenolate mofetil; TCR, tacrolimus eyedrops.

before immunosuppression, could increase its sensitivity in patients judged contraindicated for conjunctival DIF.

Nevertheless, we included patients in the study who had a strong clinical suspicion of ocular MMP, but for whom diagnostic confirmation through conjunctival DIF could not be obtained because the procedure was deemed too risky. A definitive diagnosis could be formulated only for cases where buccal DIF yielded a positive result. By contrast, for negative/non-specific buccal DIF cases, a definitive diagnosis of ocular MMP could neither be confirmed nor denied. Consequently, the possible inclusion of non-MMP cases, although rare, cannot be completely ruled out and may explain some of the negative cases.

Additionally, it is essential to note that the criteria for assessing contraindications and the perception of risk can be subjective. Many factors may influence this judgement. Clinical factors such as the extent of inflammation and the duration of untreated inflammation undoubtedly have a significant impact. But other, less measurable factors might also play a role. Patient-related comorbidities, the clinician's technical expertise, the complexity of the procedures, and logistical challenges of sample processing are just a few examples.

Moreover, the presence of uncontrolled conjunctival inflammation, which may discourage clinicians from performing conjunctival DIF, is likely to be reduced following therapy. It could therefore be reasonably argued that conjunctival biopsies in our study may have been obtained during phases of controlled disease activity. However, in two out of five patients, when oral DIF was sampled, symblepharon was present, despite controlled disease related to prolonged immunosuppressive therapy. The presence of symblepharon or cicatrization after immunosuppression might persistently represent an obstacle to considering conjunctival biopsy. In other scenarios, the long-term effects of immunosuppression should not be underestimated. Although we could not identify specific data regarding the impact of prolonged immunosuppression on the positivity rate of conjunctival biopsies, both the current findings and the available literature suggest that its effects may be comparable.

The very low rate of BP180/BP230 ELISA positive patients (only one out of nine [11%], BP180 44 U/mL, BP230 88 U/mL, cut-off 20 U/mL) suggests that oral DIF is superior to available ELISA tests; although, the latter is apparently more attractive because more easily available and does not require collaboration with other medical specialties (i.e., oral medicine specialists). This is in agreement with previous reports by Dart et al. suggesting that serological analysis of anti-BP180 and BP230 is of limited value as a surrogate diagnostic marker for conjunctival DIF-negative/DIF-unavailable ocular-only MMP. Despite the possible inclusion of non-MMP patients, which could be responsible for negative ELISA results, the lower levels of circulating antibodies in MMP and the absence of ocular-specific markers, such as integrin β4, in available panels more likely explain the present results [11].

A panel including a wider range of serological investigations (i.e., indirect immunofluorescence on salt-split skin, the search

27686566, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jvc2.70238 by Area Sistemi Dipart & Doc, Wiley Online Library on [26/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

for anti-laminin 332 antibodies by biochip analysis or immunoblotting, the search for anti-collagen VII antibodies) might have been interesting to test, but was unavailable at our institution. However, this scenario reflects the diagnostic challenge commonly found in clinical practice. Moreover, results from previous studies have shown that immunopathologic evidence of MMP in ocular-only DIF-MMP patients was difficult to achieve even with the inclusion of wider serological panels [11].

6 | Conclusions

Collectively, our results suggest that in patients with suspected ocular pemphigoid at risk of developing adverse events after conjunctival biopsy, the choice of buccal mucosa as substrate for DIF is a very attractive procedure for diagnosis confirmation and should be considered as early as possible in the diagnostic work up. Indeed, if performed early, the risk of false negatives related to ongoing immunosuppressive treatment could be reduced, and with very limited invasiveness, a significant number of patients could receive histological confirmation otherwise difficult to achieve. Nevertheless, this procedure should not replace conjunctival DIF biopsy, which remains the gold standard for diagnosing ocular MMP.

Author Contributions

Andrea Gabusi: conceptualization, investigation, data curation, writing – original draft preparation. **Michelangelo La Placa:** validation, writing – review and editing. **Davide Bartolomeo Gissi:** supervision, methodology, validation, writing – review and editing. **Federica Filippi:** investigation, data curation, writing – review and editing. **Camilla Loi:** investigation, data curation, writing – review and editing. **Cosimo Misciali:** investigation, writing – review and editing. **Federico Bardazzi:** supervision, methodology, validation, writing – review and editing.

Ethics Statement

All patients in this manuscript have given written informed consent for participation in the study and the use of their de-identified, anonymized, aggregated data and their case details (including photographs) for publication. Reviewed and approved by Local Ethical Committee (Code OBLI01).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. E. Schmidt and D. Zillikens, "Pemphigoid Diseases," *Lancet* 381 (2013): 320–332, https://doi.org/10.1016/S0140-6736(12)61140-4.

2. V. P. J. Saw and J. K. G. Dart, "Ocular Mucous Membrane Pemphigoid: Diagnosis and Management Strategies," *Ocular Surface* 6 (2008): 128–142, https://doi.org/10.1016/s1542-0124(12)70281-1.

3. E. Schmidt, H. Rashid, A. V. Marzano, et al., "European Guidelines (S3) on Diagnosis and Management of Mucous Membrane Pemphigoid, Initiated by the European Academy of Dermatology and Venereology – Part II," *Journal of the European Academy of Dermatology and Venereology* 35 (2021): 1926–1948, https://doi.org/10.1111/jdv.17395.

4. H. S. Ong, J. F. Setterfield, D. C. Minassian, et al., "Mucous Membrane Pemphigoid With Ocular Involvement," *Ophthalmology* 125 (2018): 496–504, https://doi.org/10.1016/j.ophtha.2017.10.004.

5. S. Moussa, M. Tong, M.-C. Robert, M. Harissi-Dagher, S. Ahmad, and S. Jabbour, "Survey: Preferred Practice Patterns in the Diagnosis of Mucous Membrane Pemphigoid Amongst Cornea Specialists," *European Journal of Ophthalmology* 34 (2024): 112–118, https://doi.org/10.1177/11206721231178110.

6. T. Margolis, "Evidence-Based Insights Into the Utility of Conjunctival Biopsy in Mucous Membrane Pemphigoid," *Ophthalmology* 125 (2018): 474–475, https://doi.org/10.1016/j.ophtha.2018.01.014.

7. B. Jakubowska, E. Pietrzyk, P. Maciejewicz, C. Kowalewski, and K. Wozniak, "Diagnostic and Prognostic Values of Conjunctival and Oral Biopsies Analyzed by Direct Immunofluorescence in Patients With Mucous Membrane Pemphigoid," *Frontiers in Medicine* 10 (2023): 1257288, https://doi.org/10.3389/fmed.2023.1257288.

8. S. N. Lopez, J. Cao, S. Casas de Leon, and A. R. Dominguez, "Utility of Direct Immunofluorescence Using Buccal Mucosal Biopsies in Those With Suspected Isolated Ocular Mucous Membrane Pemphigoid," *Ophthalmology* 129 (2022): 1171–1176, https://doi.org/10.1016/j.ophtha.2022.06.001.

9. M. E. Kubin, L. Hellberg, and R. Palatsi, "Glucocorticoids: The Mode of Action in Bullous Pemphigoid," *Experimental Dermatology* 26 (2017): 1253–1260, https://doi.org/10.1111/exd.13408.

10. M. M. Phiske, G. Khullar, J. K. Padhiyar, A. Hosthota, and D. Chatterjee, "Direct Immunofluorescence Demystified: Essential Insights and Recent Advances for Dermatologists," *Indian Journal of Dermatology, Venereology and Leprology* 90 (2024): 1–10, https://doi.org/10.25259/IJDVL_95_2024.

11. J. Dart, J. Setterfield, R. W. Groves, et al., "Autoantibody Detection for Diagnosis in Direct Immunofluorescence-Negative Mucous Membrane Pemphigoid," *Ophthalmology* 128 (2021): 372–382, https://doi.org/10.1016/j.ophtha.2020.07.052.

27686566, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jvc2.70238 by Area Sistemi Dipart & Doc, Wiley Online Library on [26/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License