Conjunctival Biopsy Site in Mucous Membrane Pemphigoid

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• PURPOSE: **To investigate if there is an association between the location of the conjunctival biopsy site (lesional, perilesional, or nonaffected) and the result of the direct immunofluorescence (DIF) test in patients with suspected mucous membrane pemphigoid (MMP) involving the ocular surface.**

• DESIGN: **Retrospective case series.**

• METHODS: **Records of patients with clinically suspected ocular MMP were reviewed to determine the location of the conjunctival biopsy. Conjunctival biopsy locations were defined as "lesional," "perilesional," and "nonaffected" conjunctiva. The DIF was considered positive when there was deposition of at least 1 of either IgM, IgG, IgA, or C3 at the basement membrane of the specimen; nondiagnostic when only fibrinogen was found at the same location; and negative when none of these features were present.**

• RESULTS: **The records of 41 patients were analyzed. Of these, 32 were eligible to be included in the study. Biopsies were lesional in 22% of cases (7/32), perilesional in 22% (7/32), and from nonaffected conjunctiva in 56% (18/32). DIF results were positive in 14% of lesional biopsies, in 86% of perilesional biopsies, and in 17% of those from nonaffected conjunctiva ($P = .003$). Perilesional biopsies gave higher positive DIF than lesional biopsies ($P = .029$).**

• CONCLUSIONS: **Perilesional conjunctival biopsies are associated with an increase in positive DIF results. These results support the need to sample perilesional conjunctival tissue in patients with suspected MMP.** **(Am J Ophthalmol 2020;216:1–6. © 2020 Elsevier Inc. All rights reserved.)**

MUCOUS MEMBRANE PEMPHIGOID (MMP) IS A RARE systemic immune-mediated disease that can affect several mucous membranes including the oral cavity, eye, nose, respiratory tract, and gastrointestinal tract. Ocular involvement presents as a chronic, relapsing-remitting bilateral cicatrizing conjunctivitis with progressive conjunctiva fibrosis, symblepharon formation, loss of the fornices, and entropion. This may lead to corneal ulceration and opacification with loss of vision that can cause bilateral blindness in 20% of cases.[1–4] MMP is characterized by the presence of autoantibodies that recognize and react against antigens expressed at the basement membrane zone (BMZ) in the epithelial-subepithelial junction of mucous membranes,[5] and this autoimmune reaction leads to inflammation and subsequent scarring of the involved areas.

The diagnosis of MMP involves a combination of clinical, histologic, and immunopathologic features.[6] The first international consensus on MMP concluded that both clinical findings and direct immunopathology results are essential to establish a diagnosis of MMP.[6] A direct immunofluorescence (DIF) test is considered the gold standard for the diagnosis of MMP.[6] DIF is able to detect deposition of immunoglobulins (IgG, IgA, and/or IgM) and/or complement (C3) in the epithelial BMZ.[6] It has a reported sensitivity of 70%-80%.[7–11] Although repeated and the simultaneous sampling of several sites has been advocated to increase the diagnostic sensitivity of DIF,[12,13] it would be preferable to avoid repeated biopsies of the conjunctiva and to therefore increase the yield of the initial biopsy.

Although it is well established that in cases of oral and/or skin involvement, biopsies should be taken from perilesional sites,[6] it is not known whether the same applies in cases of ocular involvement. In addition, it is not clear which area of the ocular surface should be sampled, particularly as sensitivity of DIF test performed in conjunctival biopsies can be as low as 30%.[14] It has been suggested that this reflects differences in the reactivity of conjunctival samples to immunofluorescence testing.[15] The purpose of our study was to evaluate if there was an association between the location of the conjunctival biopsy (lesional, perilesional, or nonaffected) and the DIF result.

METHODS

WE CONDUCTED A RETROSPECTIVE ANALYSIS OF PATIENTS with clinically suspected MMP who underwent

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conjunctival biopsy from March 2014 to February 2019 at The Royal Liverpool University Hospital, Liverpool, United Kingdom. Patients were considered to have suspect MMP based on clinical judgment, when various combinations of conjunctival fibrosis, fornices shortening, conjunctival cicatrization, symblepharon, corneal neovascularization, corneal scarring, cicatricial blepharitis, entropion, and trichiasis were present. A note was also made of patients who had reported oral signs and/or symptoms and were assessed in the Department of Oral Medicine, Liverpool University Dental Hospital. The ocular surface location of the conjunctival biopsy was reported in the patients' electronic charts. Location of the conjunctival samples was defined as "lesional" when the sample was taken from a well-defined area of conjunctival scarring or symblepharon, "perilesional" when the sample was taken from an area of clinically uninvolved conjunctiva adjacent to a lesion, and "nonaffected" when the sample was taken from a nonaffected distant area, either from forniceal conjunctiva or bulbar conjunctiva. Conjunctival biopsies were performed in the operating theatre under local anesthesia [topical Minims proxymetacaine hydrochloride 0.5% w/v, eye drop solution (Bausch&Lomb, Dublin, Ireland), and subconjunctival lidocaine 2% (Hameln Pharmaceuticals Ltd, Gloucester, UK)]. The size of conjunctival biopsy was not specified in patients' charts. Samples were placed in Michel's transport medium (Viapath, London, UK) and sent to the St. John's Institute of Dermatology in London for further analysis. All conjunctival samples were analyzed using direct immunofluorescence to detect the deposition of IgM, IgG, IgA, and C3 at the BMZ.[12] Results of the direct immunofluorescence analysis were labeled as "positive" when there was deposition of at least 1 of either IgM, IgG, IgA, or C3 at the basement membrane of the specimen; "nonspecific" when only fibrinogen was found at the same location; and "negative" when none of these features were present. Positive results were also considered to be diagnostic, while both nonspecific and negative results were nondiagnostic. The study was approved by the Institutional Review Board. Statistical analysis was performed using the Fisher exact test to detect a difference between diagnostic (positive) and nondiagnostic (nonspecific and negative) results according to sample location (lesional, perilesional, and nonaffected conjunctiva). Data are presented as mean ± standard deviation (SD). A P value of $<.05$ was considered statistically significant. Bonferroni correction for multiple comparisons was applied when appropriate.

RESULTS

THE RECORDS OF 41 PATIENTS WHO UNDERWENT CONJUNCTIVAL biopsy for suspect MMP were available for analysis. Mean age at the time of the biopsy was 74 ± 12.1 years and 27 patients (66%) were female. Of the 41 patients, 9 were excluded from the study because no information was available on the location of the biopsy or because no epithelium was present in the specimen. A total of 32 patients were included in the analysis. The 32 patients analyzed (22 female) had a mean age of 72 ± 12 years. Nine patients also had oral symptoms, of whom 6 also had oral DIF biopsies. The demographics and baseline clinical details of the study population are shown in Table 1. Biopsies were lesional in 22% of patients (7/32), perilesional in 22% (7/32), and from nonaffected conjunctiva in 56% (18/32). Of these, 19% were from the bulbar conjunctiva and 37% from the inferior forniceal conjunctiva. Overall, 10 of 32 (31%) biopsies gave a positive DIF test (3 of these patients also had an oral biopsy, which gave a positive DIF result in 1 case and negative DIF result in the remaining 2). Thirteen of the 32 patients (41%) had a nonspecific DIF result and 9 of 32 (28%) a negative DIF result. Of these 22 patients, 3 also had an oral biopsy and these all gave a negative DIF result.

TABLE 1. Demographics and Clinical Details of the Study Population at Baseline

Age (y), mean ± SD	72 ± 12
Sex	
Male	10 (31%)
Female	22 (69%)
Clinical signs	
Conjunctival fibrosis and/or fornix shortening	10 (31%)
Conjunctival cicatrization	14 (44%)
Symblepharon	16 (50%)
Corneal neovascularization, epitheliopathy, and/or scarring	11 (34%)
Cicatricial blepharitis and trichiasis	9 (28%)
Extraocular involvement	
Oral mucosa	9 (28%)
Skin	2 (6%)

Results from lesional biopsies were positive in 14% of cases (1/7), nonspecific in 57% (4/7), and negative in 29% (2/7). Perilesional biopsies were positive in 86% of cases (6/7) and nonspecific in 14% (1/7); none were negative. Biopsies taken from areas of nonaffected conjunctiva were positive in 17% (3/18) (17% for bulbar conjunctiva and 17% from forniceal conjunctiva), nonspecific in 44% (8/18) (50% of bulbar conjunctival samples and 42% of forniceal conjunctival samples), and negative in 39% (7/18) (33% and 42% for the bulbar and forniceal conjunctiva, respectively).

There was a significant difference in DIF from samples taken from lesional, perilesional, and nonaffected conjunctiva ($P = .003$) (Table 2). DIF from perilesional biopsies was more informative than from lesional sites ($P = .029$) (Table 2).

After a mean follow-up of 1.93 ± 1.4 years 6 patients were diagnosed as non-MMP, while the remaining 26

TABLE 2. Data on Direct Immunofluorescence Results Detailed for Biopsy Site and Biopsy Result

	Suspected MMP at the Time of Biopsy			
Biopsy Site	Positive	Nonspecific	Negative	
Overall (n = 32)	10/32 (31%)	13/32 (41%)	9/32 (28%)	
Lesional (n = 7)	1/7 (14%)	4/7 (57%)	2/7 (29%)	$P = .003$
Perilesional (n = 7)	6/7 (86%)	1/7 (14%)	0/7 (0%)	
Nonaffected conjunctiva (n = 18)	3/18 (17%)	8/18 (44%)	7/18 (39%)	
	Definite/Suspected MMP After Follow-up			
Biopsy Site	Positive	Nonspecific	Negative	
Overall (n = 26)	10/26 (38.5%)	10/26 (38.5%)	6/26 (23%)	
Lesional (n = 6)	1/6 (17%)	3/6 (50%)	2/6 (33%)	$P = .002$
Perilesional (n = 6)	6/6 (100%)	0/6 (0%)	0/6 (0%)	
Nonaffected conjunctiva (n = 14)	3/14 (21.5%)	7/14 (50%)	4/14 (28.5%)	

MMP = mucous membrane pemphigoid.

The top half of the table refers to the whole study population (n = 32) and the bottom half refers to the analysis of patients (n = 26) who were still considered to be affected by MMP after follow-up, either definite or suspect MMP. Data are presented as proportions and percentages. Fisher exact test *P* values are shown.

patients were still considered to have MMP. The diagnosis was made when an alternative cause of cicatricial conjunctivitis was considered to potentially explain the clinical ocular findings. Patients in whom no alternative diagnosis could be made were still considered to be affected by MMP and these latter cases were defined as either definite MMP, if the DIF test confirmed the diagnosis, or suspected MMP, if DIF result was negative. One patient in the suspect MMP group had lichen planus of the mouth and 1 patient had Bowen disease of the skin. Analysis in this group of patients showed that overall, of 26 biopsies, 10 were positive (38.5%), 10 nonspecific (38.5%), and 6 negative (23%). Lesional biopsies were positive in 1 of 6 cases (17%) and either nonspecific (3 of 6) or negative (2 of 6) in 5 of 6 (83%). Perilesional biopsies were positive in 6 of 6 cases (100%) and biopsies from nonaffected conjunctiva were positive in 3 of 14 (21.5%) and either nonspecific (7/14) or negative (4/7) in 11 of 14 (78.5%) ($P = .002$) (Table 2 and Figure).

DISCUSSION

WE INVESTIGATED THE ASSOCIATION BETWEEN THE SITE OF conjunctival biopsy and the DIF result in patients with clinically suspected MMP. Although there is good evidence of the need for perilesional biopsy in patients with MMP with oral and/or skin involvement, this has not been investigated in biopsies from the conjunctiva of patients with ocular involvement.[6] Results from our study support the need to sample perilesional conjunctival tissue in patients with suspected MMP in order to increase the sensitivity of DIF on conjunctival samples. The current diagnosis of MMP is based on the first international consensus criteria, according to which both clinical findings and direct immunopathology results are essential to establish the diagnosis.[6] Although it is recognized that DIF has limited sensitivity and specificity in MMP,[4,6] it provides useful diagnostic information to aid an appropriate management plan. Oral mucosa and skin biopsies have been shown to have on average 70%-80% DIF-positive results.[9,10,16] In contrast, results from conjunctival samples are lower compared to other sites and also vary widely both within and between studies, from 30% to 80%.[12,14,17,18] Since it is well accepted that extraocular sites biopsies have higher sensitivity to detect the disease, in cases of MMP with extraocular involvement, biopsies should be taken from a nonocular site.[6] When there are only ocular manifestations, it has been suggested that the diagnosis of MMP should be made, even in the absence of a positive DIF result, when clinical signs are strongly suggestive of the disease and other causes of cicatricial conjunctivitis have been excluded.[4,19,20]

It has been suggested that one of the reasons why the DIF test sensitivity is lower in conjunctival tissue is that the conjunctiva might react differently with DIF.[15] We would suggest that this low rate of positive results may reflect a mixture of conjunctival samples taken from different ocular surface locations. In addition, inflammatory nonspecific results, such as the presence of a fibrinogenous band at the basement membrane zone, have been reported with higher frequencies in conjunctival samples and have also been reported to be a normal finding in conjunctival specimens.[21] The highest percentage of DIF-positive results in conjunctival samples has been reported by Thorne and associates,[12] in a large retrospective study conducted in 2004. They found a positive DIF result of approximately 80%,

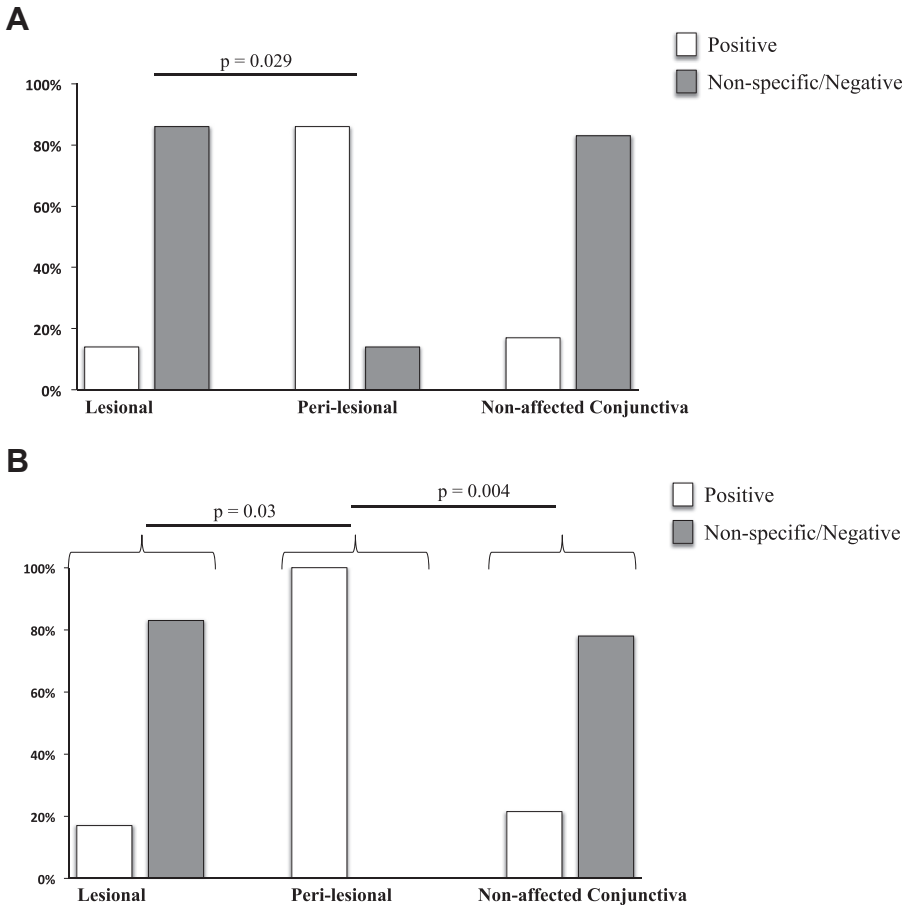

FIGURE. Graphs showing percentages of diagnostic (positive) vs nondiagnostic (nonspecific and negative) results in lesional, perilesional, and nonaffected conjunctival biopsies. (A) Results refer to the whole study population analyzed (n = 32). (B) Results refer to the analysis of patients who were still considered to be affected by mucous membrane pemphigoid after a mean follow up of 1.93 ± 1.4 years (n = 26). Fisher exact test *P* values are shown in the graph. Bonferroni corrected *P* values for multiple comparisons when appropriate.

collecting the specimen from the inferior forniceal conjunctiva of an affected eye, but unfortunately they did not specify whether they were taken from lesional, perilesional, or nonaffected areas.[12]

Although simultaneous multiple biopsies are suggested to increase the sensitivity of DIF results,[13] a recent study by Shimanovich and associates showed that more than 30% of simultaneous multiple biopsies presented with discordant results. This means that different areas collected from the same patient and at the same time reacted differently using DIF. It can be argued that these findings were due to poor biopsy site selection, either too close or too far from a lesional area, or due to an uneven distribution of mucosal autoantibodies.[13] The inflammatory process in lesional areas might alter the immunoreactants and although it is considered that autoantibodies potentially involve the entire body's mucosa,[11] they may be absent at distant sites.[13] DIF results might be more variable on the basis of sample location than previously thought and a single negative DIF result cannot exclude MMP.[13] Though repeated sampling has therefore been advocated,[12,13] it would be preferable to avoid repeated conjunctival injury and maximize the yield of the biopsy.

In our study, DIF sensitivity was 31%, which is on the low side of that reported in the literature. This, however, increased to 38% after having excluded patients who were revealed not to have MMP on the follow-up. One of the explanations for our percentage might be that most of our conjunctival biopsies were taken from nonaffected areas, thus probably including areas with absence of autoantibodies. We acknowledge that 1 of the limitations of our study is that it is a retrospective analysis and has a small sample size, and we cannot exclude that more MMP patients were in the group that received perilesional biopsies by chance. Nevertheless, at the time of biopsy all patients had the same pretest probability of being positive to the DIF test, which would support the findings. Thus far, only 1 study has compared the DIF result with the biopsy site

in ocular MMP. Mehra and associates[15] reported that while there were no differences in the presence of IgA, IgM, C3, and fibrinogen between lesional and nonlesional conjunctival biopsies, the sensitivity of IgG, which is the most common finding in MMP,[22–24] was double in nonlesional samples compared to lesional ones, reaching values of around 40%.[15] Although they did not compare perilesional and lesional and nonlesional sites, this does point to the importance of the biopsy site in determining the DIF test result.[15] Ocular MMP is a systemic disease, and extraocular involvement can affect more than 80% of cases at the time of diagnosis.[12] There is multiple evidence to support the need for systemic immunosuppression treatment[25–28] in patients with MMP, and therefore DIF results have important clinical implications in patient management.[20] Labowsky and associates reported that patients with negative DIF results were less likely to receive systemic immunosuppressive drug therapy and had, on average, shorter follow-up time.[20] This is an important observation that underlies the need to improve detection of a positive DIF result and thus provide optimal care for these patients. In conclusion, we found that perilesional conjunctival biopsies in ocular MMP patients increase the sensitivity of DIF testing. The findings of our study would support a recommendation that, in patients with clinically suspected MMP, biopsies should be taken from perilesional sites.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

GIULIA COCO: CONCEPTUALIZATION, METHODOLOGY, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft, Writing - review & editing. **Vito Romano:** Conceptualization, Methodology, Writing - review & editing. **Nardine Menassa:** Investigation, Writing - review & editing. **Davide Borroni:** Investigation, Writing - review & editing. **Katja Iselin:** Investigation, Writing - review & editing. **Daniel Finn:** Investigation, Data curation, Writing - review & editing. **Gustavo S. Figueiredo:** Investigation, Writing - review & editing. **Filofteia Tacea:** Investigation, Writing - review & editing. **Elizabeth Anne Field:** Methodology, Writing - review & editing. **Sajjad Ahmad:** Methodology, Writing - review & editing. **Stephen B. Kaye:** Conceptualization, Methodology, Writing - review & editing, Supervision.

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