


ORIGINAL ARTICLE

Direct immunofluorescence is of limited utility in patients with low clinical suspicion for an oral autoimmune bullous disorder

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Abstract

Objectives: Oral autoimmune bullous disorders show clinical overlap with diseases such as lichen planus and others that may cause desquamative gingivitis. As direct immunofluorescence is expensive, we sought to determine if routine histology alone would be sufficient to distinguish between oral autoimmune bullous disorders and mimics.

Methods: We searched the records for patients with a suspected oral autoimmune bullous disorder who underwent biopsies for concurrent routine histologic evaluation and direct immunofluorescence and who had at least one follow-up visit. Cases were separated into high and low suspicion subgroups based on clinical findings.

Results: Within 148 cases, the sensitivity of routine histology alone was 0.810, with a negative predictive value of 0.889. However, the specificity was 0.989 with a positive predictive value of 0.979. Of the high suspicion cases, 57 (47.1%) were found to be consistent with an oral autoimmune bullous disorder, with a total of 11 histologic false negatives. 8 cases, all in the high suspicion subgroup, showed indeterminate direct immunofluorescence results. There were no histologic false negatives or inconclusive direct immunofluorescence results in the low suspicion subgroup.

Conclusions: In patients with a low clinical suspicion for an oral autoimmune bullous disorder, it is reasonable and more cost-effective to evaluate the lesion with routine histology alone.

KEYWORDS

clinical risk stratification, direct immunofluorescence, immunobullous, lichen planus

1 | INTRODUCTION

Oral autoimmune bullous disorders (OABD), including mucous membrane pemphigoid (MMP), pemphigus vulgaris (PV), paraneoplastic autoimmune multiorgan syndrome/paraneoplastic pemphigus (PAMS/PNP), and others, can show considerable clinical overlap with diseases such as oral lichen planus (OLP) and other disorders that may cause desquamative or erosive gingivitis and oral ulcers

(Yih, Maier, Kratochvil, & Zieper, 1998). Although conventionally six patterns of OLP are recognized (Cheng, Gould, Kurago, Fantasia, & Muller, 2016), clinically, only three are readily distinguishable: hyperkeratotic/reticular (Figure 1), erythematous/erosive/atrophic, and ulcerative (Park, Hurwitz, & Woo, 2012). A bullous pattern has been described but is rarely seen, as oral bullae rupture due to trauma, forming erythematous/erosive lesions. OLP typically involves the buccal mucosa, gingiva, and tongue in a bilateral and symmetric fashion. When OLP presents with involvement of the gingiva or with a predominantly ulcerative or erosive pattern (Figure 2), the

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differential diagnosis can include OABD such as MMP (Figure 3) and PV (Figure 4). Furthermore, OLP and OABD occur in similar patient populations, namely females in the sixth and seventh decades of life (Laskaris, Sklavounou, & Angelopoulos, 1982; Laskaris, Sklavounou, & Stratigos, 1982). Although the symptoms and signs of OLP and MMP can often be controlled with topical corticosteroids, individuals with PV invariably require systemic therapy (Heelan et al., 2014; Lamey et al., 1992; Sultan, Villa, Saavedra, Treister, & Woo, 2017). Additionally, patients with the cicatricial variant of MMP frequently develop ocular involvement (Messmer, Hintschich, Partsch, Messer, & Kampik, 2000), and accurate diagnosis is critical to prevent serious sequelae such as loss of vision.

Both cutaneous and OLP exhibit characteristic changes on routine histology including hyperkeratosis or parakeratosis, acanthosis or atrophy, vacuolar change of the basal epithelial layer, "saw-tooth" rete ridges, Civatte bodies, and a lymphocytic band at the interface (Figure 5). Sub-epithelial clefting is often noted in OLP, often termed the Max-Joseph space (Aminzadeh, Jahanshahi, & Ahmadi, 2013), and may mimic MMP, and DIF is helpful in such cases. Other lichenoid processes, such as lichenoid drug reaction



FIGURE 1 Oral lichen planus exhibiting well-formed buccal reticulation [Colour figure can be viewed at wileyonlinelibrary.com]



FIGURE 2 Oral lichen planus presenting as diffuse desquamative gingivitis but with reticulation visible in the posterior third of the left cheek as well as the vestibular maxillary gingiva [Colour figure can be viewed at wileyonlinelibrary.com]



FIGURE 3 Pemphigus vulgaris exhibiting diffuse bullae, ulceration, and erythema [Colour figure can be viewed at wileyonlinelibrary.com]



FIGURE 4 Mucous membrane pemphigoid presenting as diffuse desquamative gingivitis [Colour figure can be viewed at wileyonlinelibrary.com]

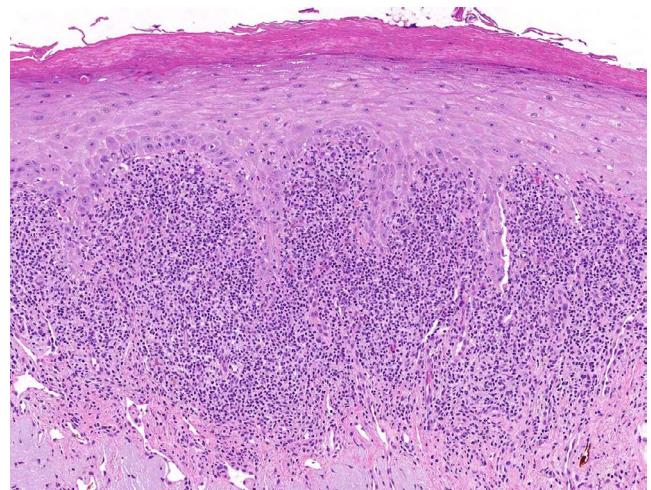


FIGURE 5 Characteristic histology of lichen planus includes hyperkeratosis, acanthosis (some cases show atrophy), "saw-tooth" rete ridges, basal cell degeneration, Civatte bodies, and a band of lymphocytes at the interface (H&E, $\times 100$) [Colour figure can be viewed at wileyonlinelibrary.com]

or contact-mediated lichenoid hypersensitivity reactions, chronic graft-versus-host disease (cGVHD), lupus erythematosus (LE), and others can show histologic overlap with OABD. Furthermore, histologic patterns on standard hematoxylin and eosin (H&E) sections can be non-specific or subtle in many cases of OABD.

For these reasons, it is commonly accepted that cases suspicious for OABD require DIF for definitive diagnosis, and OLP and other lichenoid processes exhibit a non-specific pattern of DIF, ruling out OABD. In fact, the presence of specific immune deposits at the basement membrane zone is considered a major diagnostic criterion for MMP (Chan et al., 2002). Additionally, a positive DIF result or the serological detection of autoantibodies directed against epithelial surface antigens is considered necessary for a diagnosis of pemphigus (Murrell et al., 2018). In the current environment of growing demand to curb increases in healthcare costs while upholding high standards of patient care, we sought to determine if hematoxylin and eosin (H&E) evaluation alone would, in selected cases, be sufficient to distinguish between OABD and clinical mimics, keeping in mind that definitive characterization is sometimes more reliably arrived at from the clinical presentation and subsequent follow-up.

2 | MATERIALS AND METHODS

This retrospective study was approved by the Brigham and Women's Hospital (BWH) Institutional Review Board (protocol number 201700010) and was performed in accordance with the Declaration of Helsinki. We searched the BWH pathology records for patients with suspected OABD who underwent biopsies for concurrent H&E and DIF studies and who had at least one follow-up visit at our hospital during the period from 1993 to 2016. Cases that had insufficient tissue, such as the lack of epithelium on either the H&E or DIF preparation, were excluded. Upon review of the medical record, cases were separated into high suspicion (HS) and low suspicion (LS) subgroups based on clinical findings. Physical examination findings were

recorded as the presence of reticulation, erythema, or ulceration, or a combination of the three, and any additional descriptors such as bullae or vesicles. Criteria for LS and HS cases are presented in Table 1. HS cases included those with either desquamative gingivitis or mucosal ulceration without evidence of reticulation (for additional criteria see Table 1). Patients needed to only satisfy one criterion in either the low clinical suspicion or high clinical suspicion categories to qualify for the respective group. Specifically, if a patient presented with clinical findings and history consistent with classic oral lichen planus or recurrent aphthous stomatitis, and/or had concomitant diseases associated with either condition, that patient was considered to be in the low suspicion group. Alternatively, if a patient's physical examination revealed either desquamative gingivitis or oral ulcers not consistent with oral lichen planus or RAS and/or there were mucocutaneous signs and symptoms concerning for MMP, PV, or PNP/PAMS, they were deemed to be highly suspicious for OABD.

On routine H&E evaluation, cases were diagnosed as compatible or characteristic of OABD or not compatible with OABD based on commonly accepted criteria. For example, PV characteristically shows suprabasal acantholysis and intraepithelial blister formation (Figure 6). MMP classically causes a subepithelial split with preservation of basal cells that can often be quite subtle (Figure 7). DIF findings were categorized as either diagnostic of OABD, negative for a specific pattern of immunoreactivity, or inconclusive. Characteristic patterns of DIF included linear deposition of IgG and/or C3 along the basement membrane zone (BMZ) for MMP and a fish net-like pattern of intercellular IgG and C3 within the epithelium for PV. A definitive diagnosis was then assigned to the patient based on biopsy results, DIF, correlation with clinical findings, laboratory results (including indirect immunofluorescence), and, in some cases, re-biopsy. Sensitivity, specificity, positive predictive value, and negative predictive value of H&E tissue examination were then calculated based on standard 2 × 2 contingency tables within the LS and HS subgroups for cases that were definitively classified as OABD or non-OABD (all but one case). Values for all cases regardless of clinical suspicion were also calculated.

TABLE 1 Criteria used to determine level of suspicion for OABD

Low clinical suspicion	High clinical suspicion
Bilateral, symmetric reticulation with or without erythema and ulceration (consistent with OLP)	Desquamative gingivitis without reticulation
Concomitant diseases associated with oral lichenoid lesions, such as LE	Focal or multifocal ulceration without reticulation
1–5 discrete ulcers, <1 cm, with a clinical history of episodic ulcers since childhood (consistent with RAS)	Intact or collapsed bulla(e) present without reticulation
Concomitant diseases associated with RAS, such as Crohn disease	Mucocutaneous signs and symptoms (e.g., ocular, nasal, genital involvement) suggestive of MMP, PV, or PNP/PAMS

Abbreviations: LE, lupus erythematosus; MMP, mucous membrane pemphigoid; OLP, oral lichen planus; PAMS/PNP, paraneoplastic autoimmune multiorgan syndrome/paraneoplastic pemphigus; PV, pemphigus vulgaris; RAS, recurrent aphthous stomatitis.

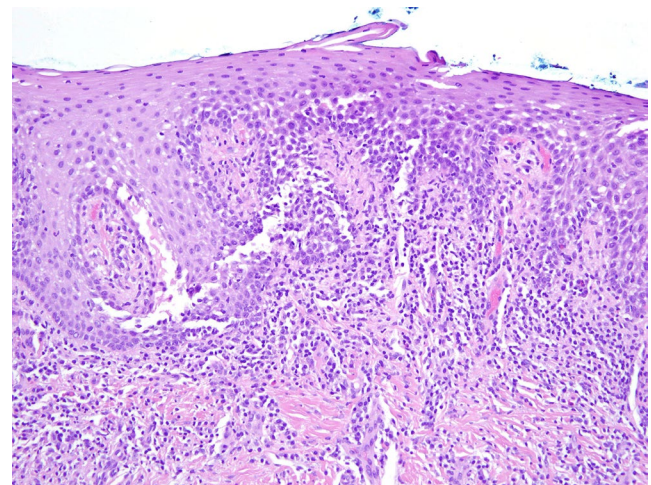


FIGURE 6 Characteristic histopathology of pemphigus vulgaris demonstrating suprabasal acantholysis (H&E, ×100) [Colour figure can be viewed at wileyonlinelibrary.com]

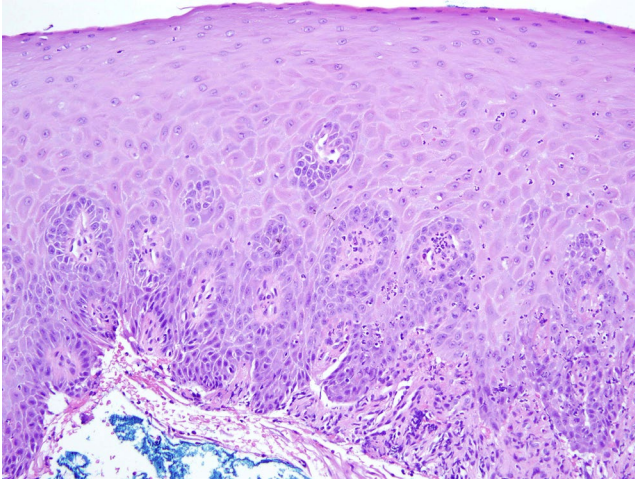


FIGURE 7 Mucous membrane pemphigoid showing preservation of the basal cells and subtle subepithelial clefting (H&E, $\times 100$) [Colour figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

There were 148 cases, with a mean age of 56 years (Table 2); there were 103 females (69.6%) and 45 males (30.4%). One hundred and thirty-three patients were definitively diagnosed based on H&E histopathology, DIF, and clinical presentation. The most common diagnoses were OLP or lichenoid hypersensitivity reaction (47), MMP (27), and PV (26). Of the 15 cases that did not receive a definitive diagnosis, 14 cases were determined to be non-OABD by DIF results. In the one remaining case, the differential diagnosis was either cGVHD or PAMS/PNP in a patient with a history of chronic lymphocytic leukemia status post-allogeneic bone marrow transplantation.

In this case, DIF showed a pattern of intercellular deposition of IgG, but none within basement membrane zone.

There were 27 LS and 121 HS cases (Table 3). In the LS subgroup, just one case had a positive DIF study that was interpreted on H&E as non-specific chronic inflammation with eosinophils and focal subepithelial clefting, raising suspicion for MMP. DIF showed strong linear IgG and C3 along the BMZ, consistent with MMP. As focal findings characteristic of MMP were indeed present in this case, it was considered to be a true positive histologic result. Of the 26 remaining cases in the LS subgroup, H&E evaluation was inconsistent with OABD and DIF studies were all negative for specific immune deposits. Of these cases, 14 of 26 were assigned a definitive diagnosis of OLP or lichenoid hypersensitivity reaction. Two of these cases were associated with systemic lupus erythematosus (SLE; Table 3). Although OLP-like lesions can be seen as a manifestation of SLE, characteristic findings of lupus were not seen on DIF in either case. One additional case was diagnosed as recurrent aphthous stomatitis (RAS) associated with Crohn disease. Two cases were diagnosed as cGVHD. Seven additional cases were assigned a definitive diagnosis (after correlation with clinical findings, other clinical tests, and consideration of pathology reports and other laboratory data) that included various entities (Table 3). The two remaining cases were not assigned a final diagnosis but were considered to be non-OABD.

Of the HS cases, 57 of 121 (47.1%) were found to be consistent with OABD. This included 26 cases of MMP and 26 cases of PV. There were three cases of PAMS/PNP, two of which were associated with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), and one with a gastrointestinal neuroendocrine tumor. There was one case of pemphigoid gestationis and one case of linear IgA disease; both patients had skin lesions in addition to oral lesions. Importantly,

TABLE 2 Demographic information of the patient cohort separated into OLP and other non-OABD cases, OABD cases, and those with an undetermined diagnosis

	OLP and other non-OABD	OABD	Undetermined, but non-OABD	Undetermined	Total
Number of cases	75	58	14	1	148
Average age (y)	55	56	53	76	56
Number female	58 (77.3%)	38 (65.5%)	7 (50.0%)	0 (0%)	103 (69.6%)
Number male	17 (22.7%)	20 (34.5%)	7 (50.0%)	1 (100%)	45 (30.4%)
Number LS	24	1	2	0	27
Number HS	51	57	12	1	121
Definitive diagnosis	47 OLP 13 RAS 2 cGVHD 2 EM 11 other ^a	27 MMP 26 PV 3 PNP/PAMS 1 PG 1 linear IgA	n/a	cGVHD versus PNP/PAMS	

Abbreviations: cGVHD, chronic graft-versus-host disease; EM, erythema multiforme; HS, high suspicion; LS, low suspicion; MMP, mucous membrane pemphigoid; OABD, oral autoimmune bullous disorder; OLP, oral lichen planus; PG, pemphigoid gestationis; PNP/PAMS, paraneoplastic pemphigus/paraneoplastic autoimmune multiorgan syndrome; PV, pemphigus vulgaris; RAS, recurrent aphthous stomatitis.

^aOther diagnosis included plasma cell gingivitis, exfoliative cheilitis, hyperkeratosis not otherwise specified, osteonecrosis of the jaw (ONJ) presenting with erythematous gingiva, squamous cell carcinoma, crohn disease, methotrexate stomatitis, autoimmune progesterone dermatitis, desquamative gingivitis in the setting of celiac disease, gingivitis (periodontal disease), and herpes simplex virus infection.

**TABLE 3** Definitive diagnoses according to clinical suspicion subgroup

Definitive diagnosis	Low clinical suspicion	High clinical suspicion
OABD (total)	1	57
MMP	1	26
PV	0	26
PAMS/PNP	0	3
PG	0	1
Linear IgA	0	1
OLP and non-OABD with definitive diagnosis (total)	24	51
OLP	14 (2 associated with SLE)	33
RAS	1 (associated with CD)	12
cGVHD	2	0
EM	0	2
Other	7 (plasma cell gingivitis, exfoliative cheilitis, hyperkeratosis, ONJ with erythematous gingiva, SCC, CD, MTX-associated stomatitis)	4 (autoimmune progesterone dermatitis, desquamative gingivitis in the setting of celiac disease, non-specific gingivitis, HSV infection)
Undetermined, but non-OABD	2	12
Undetermined (OABD versus non-OABD)	0	1 (cGVHD vs. PAMS/PNP)
Total	27	121

Abbreviations: CD, Crohn disease; cGVHD, chronic graft-versus-host disease; EM, erythema multiforme; HSV, herpes simplex virus; MMP, mucous membrane pemphigoid; MTX, methotrexate; OABD, oral autoimmune bullous disorder; OLP, oral lichen planus; ONJ, osteonecrosis of the jaw; PAMS/PNP, paraneoplastic autoimmune multiorgan syndrome/paraneoplastic pemphigus; PG, pemphigoid gestationis; PV, pemphigus vulgaris; RAS, recurrent aphthous stomatitis; SCC, squamous cell carcinoma; SLE, systemic lupus erythematosus.

within this group of definitive OABD cases that were HS lesions, there were seven cases that initially showed indeterminate DIF results, namely weak or focal staining that was insufficient for a diagnosis of OABD. However, based on the clinical presentation and follow-up, and in some cases re-biopsy, these cases were eventually diagnosed as MMP (three cases), PAMS/PNP (two cases) and PV (one case). In the remaining case, the diagnosis was equivocal and the differential diagnosis included chronic GVHD and PAMS/PNP. In terms of histologic diagnosis, although one report showed squamous mucosa with no specific pathologic change, most biopsies showed non-specific chronic inflammation with no significant changes in the overlying epithelium.

Of the 50 non-OABD HS cases, 33 were diagnosed as OLP or lichenoid hypersensitivity reaction (one being to cinnamic aldehyde), one with associated conjunctival scarring; this patient had scarring OLP confirmed with conjunctival biopsy with DIF studies. Twelve HS cases were diagnosed as non-specific ulcers consistent with RAS and two cases were diagnosed as erythema multiforme. The remaining four cases were assigned miscellaneous non-OABD diagnoses (Table 3), including autoimmune progesterone dermatitis, which does not form bullae, and typically shows non-specific DIF findings but with specific antibodies detectable by indirect immunofluorescence (Farah & Shbaklu, 1971; Lee, Yoon, Yi, & Cho, 1992). Thirteen additional cases

were not assigned definitive diagnoses, even after clinical correlation and reviewer evaluation; however, 12 of these were considered to be non-OABD. One of the non-OABD HS cases showed indeterminate DIF results and was eventually diagnosed as OLP.

Altogether, there were 11 H&E and 6 DIF false negatives, all in the HS subgroup (Table 4). In two of six latter cases, the H&E diagnosis was consistent with MMP, namely subepithelial cleaving with preservation of basal cells and minimal inflammation. In the remaining four cases, the diagnosis of OABD was confirmed clinically or by repeat biopsy—two cases of MMP, the one case of pemphigoid gestationis, and one case of PV. There was one H&E false positive, which was in the high suspicion group due to the absence of reticulation on exam. This was a patient whose histology showed squamous mucosa with hyperparakeratosis and interface stomatitis without acantholysis. As there was some subepithelial separation, it was noted that the findings could represent bullous lichen planus or pemphigoid. However, DIF was negative for specific immune deposits.

In summary, the sensitivity of H&E alone (using a summative total of clinical presentation, follow-up, and DIF as the gold standard) was just 0.810 (Table 5), with a negative predictive value of 0.889. However, H&E findings were highly specific (0.989) with a high positive predictive value (0.979).

TABLE 4 H&E false negatives with corresponding DIF and definitive diagnoses

H&E diagnosis	DIF diagnosis	DIF category	Definitive diagnosis
Ulcer with acute and chronic inflammation and basal cell degeneration	No immunoreactivity	False negative	MMP (established by re-biopsy of skin with DIF)
Ulcer bed with detached benign squamous epithelium	Oral mucosa with 4 + C3 and 3 + IgG in a linear array along the BMZ consistent with MMP	True positive	MMP
Chronic inflammation with rare eosinophils	Weak granular C3 and strong linear IgG at the BMZ	True positive	MMP
Non-specific acute and chronic inflammation	Weak linear IgA and strong linear fibrinogen at BMZ	True positive	Linear IgA disease
Chronic inflammation	Linear basement membrane staining with antibodies to IgG and C3	True positive	MMP
Mild chronic inflammation	3 + linear C3 deposition and 3 + linear deposition of IgG at BMZ, focal granular deposition of IgM	True positive	MMP
Acute and chronic inflammation	Linear BMZ and intercellular deposition of 3 + IgG and 2 + C3	True positive	PAMS/PNP
Acute and chronic inflammation	Weak granular C3 and fibrin deposition along the BMZ and within dermal vessels.	True positive	Pemphigoidgestationis
Lichenoid mucositis	Partial subepithelial clefting with 1 + focal granular C3 and IgG deposition and foci of weak superficial epithelial IgG and C3 intercellular staining. Indirect immunofluorescence recommended.	Inconclusive	PAMS/PNP
No specific pathologic findings	2 + C3 and 4 + IgG in an intercellular pattern	True positive	PV
Mild chronic inflammation and scattered eosinophils	Weak to moderate patchy intercellular C3, strong intercellular IgG	True positive	PV

Abbreviations: BMZ, basement membrane zone; DIF, direct immunofluorescence; MMP, mucous membrane pemphigoid; PAMS/PNP, paraneoplastic autoimmune multiorgan syndrome/paraneoplastic pemphigus; PV, pemphigus vulgaris.

TABLE 5 Sensitivity, specificity, positive predictive value, and negative predictive value of H&E for the diagnosis of OABD

Value	All cases	High clinical suspicion	Low clinical suspicion
Sensitivity	0.810	0.807	1
Specificity	0.989	0.980	1
PPV	0.979	0.978	1
NPV	0.889	0.817	1
Prevalence of OABD	0.392	0.471	0.037

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

4 | DISCUSSION

As of this writing, at one author's institution, each skin/mucosal specimen for DIF is billed at approximately \$3,600. As such, we sought to determine which patients with findings suspicious for OABD are most likely to benefit from the use of DIF. As many cases of OLP present with a classic pattern of involvement, we hypothesized that in these LS cases, DIF would likely not prove to be cost-effective. Indeed, approximately one third of OLP patients in this study showed the typical, well-described pattern of

symmetric reticular, erythematous, or ulcerative lesions in the region of the buccal mucosa or gingiva, similar to previous reports (Eisen, 2002). These cases, in retrospect, did not require DIF to be performed.

The diagnostic utility of DIF in establishing the diagnosis of OLP has been debated. One prior study has shown that OLP patients more commonly show a linear, shaggy, or granular pattern of fibrinogen deposition in the basement membrane zone (Rogers & Jordon, 1977; Yamanaka et al., 2017). However, these findings are not specific and have also been observed at a fairly high frequency in both pre-malignant and malignant conditions (Montague, Bhattacharyya, Islam, Cohen, & Fitzpatrick, 2015). On the contrary, DIF may aid in distinguishing between the ulcerative pattern of OLP and chronic ulcerative stomatitis (Ko, Danciu, Fullen, & Chan, 2018).

Although our cohort included 11 false negative H&E diagnoses, most cases were likely the result of sampling error, perhaps related to procedural difficulty in obtaining oral biopsy specimens. Oral mucosal biopsies are technically challenging because of extreme mucosal fragility in OABD, especially at gingival sites where such lesions frequently occur and the mucosa is very thin (Arundhati, Ragunatha, & Mahadeva, 2013; Sultan et al., 2017). Interestingly, there were six cases in which DIF was interpreted as negative but after clinical follow-up, re-biopsy (of oral mucosa or skin), or indirect

immunofluorescence, these cases were revealed to be OABD. Five of six cases of false negative DIF biopsies were taken from the gingiva, which was found to be a site yielding low sensitivity in a recent report, probably because of such sampling error (Sano et al., 2008; Solomon, Helm, Stevens, Neiders, & Kumar, 2007; Sultan et al., 2017). However, it has been shown that specimens taken for DIF from non-perilesional or distant oral sites rather than perilesional tissue have a similar rate of positivity (Sano et al., 2008). Sites that yielded higher rates of positivity in this study included floor of mouth, hard palatal mucosa, lip mucosa, and ventral tongue, with punch biopsy being a more sensitive method than biopsy by scalpel (Sano et al., 2008).

Our results may be specific to our patient population and referral pattern; additionally, the retrospective nature of the study introduces possible selection bias, possibly with regard to search terms used. However, the results indicate that in patients with a low clinical suspicion for OABD, it is reasonable and more cost-effective to perform a biopsy for H&E alone in select patients. An alternative solution would be to separate a larger biopsy into two fragments or harvest two biopsies, one for formalin fixation and routine H&E histopathology, and the other in DIF transfer medium (such as Michel's transport medium) that can be stored for several days prior to processing until H&E results are obtained. In this framework, patients with histologic features suggestive or suspicious for OABD would receive DIF testing of the stored specimen after reporting of H&E results. However, if the suspicion for OABD is high, concurrent, up-front submission for DIF is recommended, as H&E alone fails to recognize a number of cases (Arundhathi et al., 2013; Yih et al., 1998). Although DIF is still considered the gold standard for the diagnosis of immunobullous disease and is necessary for definitive diagnosis (Giurdanella, Diercks, Jonkman, & Pas, 2016; "Oral features of mucocutaneous disorders", 2003), our analysis indicates that DIF is not invariably reliable, and close clinical follow up and sometimes re-biopsy may be needed for definitive diagnosis. Furthermore, a high index of suspicion warrants repeat DIF testing if routine histologic examination is non-specific or suggestive of OABD.

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CONFLICTS OF INTEREST

The authors report no potential conflicts of interest.

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REFERENCES

Aminzadeh, A., Jahanshahi, G., & Ahmadi, M. (2013). A retrospective comparative study on clinico-pathologic features of oral lichen

- planus and oral lichenoid lesions. *Dental Research Journal*, 10(2), 168. <https://doi.org/10.4103/1735-3327.113328>
- Arundhathi, S., Rangunatha, S., & Mahadeva, K. C. (2013). A cross-sectional study of clinical, histopathological and direct immunofluorescence spectrum of vesiculobullous disorders. *Journal of Clinical and Diagnostic Research*, 7(12), 2788–2792. <https://doi.org/10.7860/JCDR/2013/7019.3760>
- Chan, L. S., Ahmed, A. R., Anhalt, G. J., Bernauer, W., Cooper, K. D., Elder, M. J., ... Zone, J. J. (2002). The first international consensus on mucous membrane pemphigoid. *Archives of Dermatology*, 138(3), 370–379. <https://doi.org/10.1001/archderm.138.3.370>
- Cheng, Y. S. L., Gould, A., Kurago, Z., Fantasia, J., & Muller, S. (2016). Diagnosis of oral lichen planus: A position paper of the American academy of oral and maxillofacial pathology. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, 122(3), 332–354. <https://doi.org/10.1016/j.oooo.2016.05.004>
- Eisen, D. (2002). The clinical features, malignant potential, and systemic associations of oral lichen planus: A study of 723 patients. *Journal of the American Academy of Dermatology*, 46(2), 207–214. <https://doi.org/10.1067/mjd.2002.120452>
- Farah, F. S., & Shbaklu, Z. (1971). Autoimmune progesterone urticaria. *The Journal of Allergy and Clinical Immunology*, 48(5), 257–261. [https://doi.org/10.1016/0091-6749\(71\)90025-X](https://doi.org/10.1016/0091-6749(71)90025-X)
- Giurdanella, F., Diercks, G., Jonkman, M., & Pas, H. (2016). Laboratory diagnosis of pemphigus: Direct immunofluorescence remains the gold standard. *British Journal of Dermatology*, 175(1), 185–186. <https://doi.org/10.1111/bjd.14408>
- Heelan, K., Al-Mohammed, F., Smith, M. J., Knowles, S., Lansang, P., Walsh, S., & Shear, N. H. (2014). Durable remission of pemphigus with a fixed-dose rituximab protocol. *JAMA Dermatology*, 150(7), 703–708. <https://doi.org/10.1001/jamadermatol.2013.6739>
- Ko, E. M., Danciu, T. E., Fullen, D. R., & Chan, M. P. (2018). Chronic ulcerative stomatitis: Case series of an under-recognized entity. *Journal of Cutaneous Pathology*, 45(12), 927–932. <https://doi.org/10.1111/cup.13347>
- Lamey, P., Rees, T., Binnie, W., Wright, J., Rankin, K., & Simpson, N. (1992). Oral presentation of pemphigus vulgaris and its response to systemic steroid therapy. *Oral Surgery, Oral Medicine, and Oral Pathology*, 74(1), 54–57. [https://doi.org/10.1016/0030-4220\(92\)90215-C](https://doi.org/10.1016/0030-4220(92)90215-C)
- Laskaris, G., Sklavounou, A., & Angelopoulos, A. (1982). Direct immunofluorescence in oral lichen planus. *Oral Surgery, Oral Medicine, and Oral Pathology*, 53(5), 483–487. [https://doi.org/10.1016/0030-4220\(82\)90461-3](https://doi.org/10.1016/0030-4220(82)90461-3)
- Laskaris, G., Sklavounou, A., & Stratigos, J. (1982). Bullous pemphigoid, cicatricial pemphigoid, and pemphigus vulgaris. *Oral Surgery, Oral Medicine, and Oral Pathology*, 54(6), 656–662. [https://doi.org/10.1016/0030-4220\(82\)90080-9](https://doi.org/10.1016/0030-4220(82)90080-9)
- Lee, C. W., Yoon, K. B., Yi, J. U., & Cho, S. H. (1992). Autoimmune progesterone dermatitis. *The Journal of Dermatology*, 19(10), 629–631. <https://doi.org/10.1111/j.1346-8138.1992.tb03743.x>
- Messmer, E., Hintschich, K., Partscht, K., Messer, G., & Kampik, A. (2000). Ocular cicatricial pemphigoid: Risk factors and complications. *Ophthalmologie*, 97(2), 113–120.
- Montague, L. J., Bhattacharyya, I., Islam, M. N., Cohen, D. M., & Fitzpatrick, S. G. (2015). Direct immunofluorescence testing results in cases of premalignant and malignant oral lesions. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology*, 119(6), 675–683. <https://doi.org/10.1016/j.oooo.2015.02.478>
- Murrell, D. F., Peña, S., Joly, P., Marinovic, B., Hashimoto, T., Diaz, L. A., ...Werth, V. P. (2018). Diagnosis and management of pemphigus: Recommendations by an international panel of experts. *Journal of the American Academy of Dermatology*, (March 2017). <https://doi.org/10.1016/j.jaad.2018.02.021>

- Park, H. K., Hurwitz, S., & Woo, S. B. (2012). Oral lichen planus: REU scoring system correlates with pain. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, 114(1), 75–82. <https://doi.org/10.1016/j.oooo.2012.02.013>
- Research, Science and Therapy Committee (2003). Oral features of mucocutaneous disorders. *Journal of Periodontology*, 74(10), 1545–1556. <https://doi.org/10.1902/jop.2003.74.10.1545>
- Rogers, R. S., & Jordon, R. E. (1977). Immunopathology of oral mucosal inflammatory diseases. *Clinical and Experimental Dermatology*, 2(2), 97–107. <https://doi.org/10.1111/j.1365-2230.1977.tb01550.x>
- Sano, S. M., Quarracino, M. C., Aguas, S. C., González, E. J., Harada, L., Krupitzki, H., & Mordoh, A. (2008). Sensitivity of direct immunofluorescence in oral diseases. Study of 125 cases. *Medicina Oral, Patología Oral Y Cirugía Bucal*, 13(5), 287–291. [https://doi.org/10.1016/S1077-9108\(08\)79026-1](https://doi.org/10.1016/S1077-9108(08)79026-1)
- Solomon, L. W., Helm, T. N., Stevens, C., Neiders, M. E., & Kumar, V. (2007). Clinical and immunopathologic findings in oral lichen planus pemphigoides. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 103(6), 808–813. <https://doi.org/10.1016/j.tripleo.2006.03.020>
- Sultan, A. S., Villa, A., Saavedra, A. P., Treister, N. S., & Woo, S. B. (2017). Oral mucous membrane pemphigoid and pemphigus vulgaris—a retrospective two-center cohort study. *Oral Diseases*, 23(4), 498–504. <https://doi.org/10.1111/odi.12639>
- Yamanaka, Y., Yamashita, M., Innocentini, L. M. A., Macedo, L. D., Chahud, F., Ribeiro-Silva, A., ... Motta, A. C. (2017). Direct immunofluorescence as a helpful tool for the differential diagnosis of oral lichen planus and oral lichenoid lesions. *The American Journal of Dermatopathology*, 40(7), 491–497. <https://doi.org/10.1097/DAD.0000000000001071>
- Yih, W.-Y., Maier, T., Kratochvil, F. J., & Zieper, M. B. (1998). Analysis of desquamative gingivitis using direct immunofluorescence in conjunction with histology. *Journal of Periodontology*, 69(6), 678–685. <https://doi.org/10.1902/jop.1998.69.6.678>

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