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Desquamative gingivitis evaluation with line-field confocal optical coherence tomography may support differentiation of pemphigus from pemphigoid

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Abstract

Objectives

To characterize and compare line-field confocal optical coherence tomography (LC-OCT) features of desquamative gingivitis in patients with pemphigus vulgaris and pemphigoid and to assess lesion distribution and associations with disease activity.

Materials and methods

This single-center retrospective cross-sectional study collected clinical and LC-OCT data from 23 adults with desquamative gingivitis associated with pemphigus vulgaris (12) or pemphigoid (11). Fisher's exact tests with false discovery rate (FDR) correction and Spearman correlations were used.

Results

Pemphigus vulgaris was characterized by presumed acantholytic cells, presenting in LC-OCT as hyperreflective, gray, drop-shaped elements attached to the cleft roof and floating within the cleft and erosion (partial-thickness epithelial disruption) (12/12 vs 0/11; FDR-adjusted $p < 0.001$) and intraepithelial blisters (hyporeflective intraepithelial cleft) (8/12 vs 0/11; FDR-adjusted $p = 0.004$). Pemphigoid was characterized by a subepithelial blister filled with putative fibrin and inflammatory cells (hyporeflective subepithelial cleft containing homogeneous, mildly reflective material and small bright particles at the basement membrane zone) (11/11 vs 0/12; FDR-adjusted $p < 0.001$). Pemphigus vulgaris involved a wider range of mucocutaneous sites than pemphigoid. In pemphigus vulgaris, epithelial small bright particles were not significantly associated with clinical activity scores, disease duration, anti-desmoglein 1 (Dsg1) and anti-desmoglein 3 (Dsg3) antibody levels.

Conclusions

LC-OCT provides real-time, noninvasive imaging features that may support the differential diagnosis of pemphigus vulgaris and pemphigoid presenting as desquamative gingivitis.

Clinical relevance:

LC-OCT may serve as a useful adjunctive tool to support initial assessment and differentiation of these conditions, potentially reducing diagnostic delay and guiding earlier targeted therapy.

Introduction

Desquamative gingivitis is a descriptive clinical term encompassing erythematous, erosive, and blistering lesions of the gingiva associated with several mucocutaneous disorders [1]. Among autoimmune blistering diseases, it occurs most commonly in pemphigus vulgaris and pemphigoid, more frequently in mucous membrane pemphigoid and rarely in bullous pemphigoid [1, 2]. Although their gingival manifestations may be clinically indistinguishable, pemphigus vulgaris differs from mucous membrane pemphigoid and bullous pemphigoid in the level of tissue separation: pemphigus vulgaris results from intraepithelial acantholysis, whereas both pemphigoids are characterized by a subepithelial cleft [1, 3, 4]. Definitive diagnosis therefore relies on immunopathological examination, particularly direct immunofluorescence (DIF) of an appropriately selected perilesional or clinically unaffected oral biopsy [3–5].

Diagnostic delay remains a practical problem in desquamative gingivitis. Daltaban et al. reported a mean delay of 8.25 months in patients with pemphigus vulgaris who initially presented with desquamative gingivitis, while Caggiula et al. found mean delays of 8.0 months for pemphigus vulgaris and 10.71 months for mucous membrane pemphigoid [6, 7]. A case report of mucous membrane pemphigoid with a negative Nikolsky sign described gingival symptoms persisting for 6 months before specialist assessment, illustrating how subtle clinical findings may further complicate recognition [8]. These delays underscore the need for adjunctive tools that may support earlier differentiation and guide the diagnostic workup.

Conventional optical coherence tomography (OCT) has also been used to evaluate epithelial architecture, tissue reflectivity, and basement membrane integrity in oral mucosal biopsy specimens, further supporting the potential of optical imaging for the structural characterization of oral lesions [9]. Line-field confocal optical coherence tomography (LC-OCT) combines vertical and horizontal imaging with near-cellular resolution, enabling real-time, noninvasive visualization of tissue architecture [10, 11]. Previous LC-OCT studies in autoimmune blistering diseases have focused on cutaneous lesions and have shown that this technique can identify the level of tissue separation and provide information on blister morphology and content [10, 12]. Evidence regarding oral mucosa remains limited, and LC-OCT features of gingival pemphigus vulgaris and pemphigoid have not been directly compared [11]. Therefore, this study aimed to characterize and compare the LC-OCT features of desquamative gingivitis in patients with pemphigus vulgaris and pemphigoid and to examine lesion distribution and selected associations with disease activity.

Materials and methods

2.1. Study design and patients

Clinical data and archived LC-OCT images obtained between 1 October 2023 and 4 May 2026 were generated as part of routine clinical care. These data were accessed, extracted, and analyzed for research purposes only after approval of the study protocol by the Bioethics Committee of the Medical University of Warsaw on 4 May 2026 (approval no. KB/28/2026). This was a retrospective study.

For the present analysis, eligible participants were adults with desquamative gingivitis and evaluable LC-OCT images. The pemphigoid group comprised 10 patients with mucous membrane pemphigoid and one patient with bullous pemphigoid. Both subtypes were analyzed together because the primary comparison concerned the intraepithelial versus subepithelial level of tissue separation.

Other causes of desquamative gingivitis were outside the scope of this analysis. Exclusion criteria were an oral disorder interfering with assessment, active oral infection, systemic immunosuppressive treatment, severe systemic disease preventing participation, or inability to cooperate. Topical glucocorticosteroid treatment was permitted. Written informed consent for participation and the scientific use of clinical and imaging data was obtained from all participants. All eligible patients meeting these criteria and available by the data cut-off were included. No formal sample-size calculation was performed. Pemphigus vulgaris (~ 2.83 per million person-years) [13] and pemphigoid (~ 2 per million per year) [14] are rare autoimmune blistering diseases in which desquamative gingivitis is an even rarer presenting sign.

2.2. Clinical and laboratory assessment

A standardized form captured age, sex, symptom duration, pain visual analogue scale (VAS), Nikolsky sign, lesion morphology, and oral or extraoral distribution. Disease severity was assessed using the Oral Disease Severity Score (ODSS) in both groups [15, 16], the Pemphigus Disease Area Index (PDAI) in pemphigus vulgaris [17], and the Mucous Membrane Pemphigoid Disease Area Index (MMPDAI) as a descriptive mucosal activity measure in pemphigoid [18].

The diagnosis of pemphigus vulgaris or pemphigoid was confirmed by DIF of a buccal mucosal biopsy. Immunoglobulin G (IgG), immunoglobulin A (IgA), and complement component 3 (C3) intensity were graded from 0 to 3 for exploratory correlations. Routine testing included indirect immunofluorescence (IIF) and commercial enzyme-linked immunosorbent assays (ELISAs) for bullous pemphigoid antigen 180 (BP180), bullous pemphigoid antigen 230 (BP230), laminin 332, Dsg1 and Dsg3 autoantibodies (EUROIMMUN, Lübeck, Germany).

2.3. LC-OCT image acquisition and analysis

LC-OCT imaging was performed using the deepLive™ system (DAMAE Medical, Paris, France; axial and lateral resolutions of 1.1 and 1.3 µm, respectively; penetration depth > 400 µm; stack size 1.2 × 0.5 × 0.5 mm). Vertical and horizontal scans were acquired from one representative gingival lesion between the canines in sextant II or V. Imaging was standardized to these gingival regions to reduce site-related variability, as evidence from confocal laser endomicroscopy indicates that oral mucosal architecture and optical image quality differ across anatomical locations [19]. Integrated videodermoscopy was used to document the imaging site. Minimal probe pressure was applied to reduce imaging artifacts.

Images were presented in random order. Two evaluators (U.F., M.K.), blinded to the final diagnosis and laboratory results, independently assessed images using a predefined sheet covering epithelial, BMZ,

lamina propria, blister, and vascular features. The scoring sheet and the 19 LC-OCT features were defined before image review. Variables were binary.

2.4. Statistical analysis

Categorical data are reported as n/N (%). Where applicable, categorical variables in patients with pemphigus vulgaris versus pemphigoid were compared using Fisher's exact tests. Continuous and ordinal data are presented as medians (interquartile range, IQR). Spearman’s rho was used to assess exploratory associations between LC-OCT features and clinical or laboratory variables. Interobserver agreement was summarized as the feature-specific percentage agreement based on the two independent ratings.

The primary analysis evaluated differences in the prevalence of 19 prespecified LC-OCT features in patients with pemphigus vulgaris versus those with pemphigoid. There were no missing data for any of the variables analyzed. The resulting p-values were adjusted using the Benjamini-Hochberg procedure to control the false discovery rate (FDR), with an adjusted $p < 0.05$ considered statistically significant. Analyses of clinical distribution, videodermoscopic features, and correlations were exploratory and were not adjusted for multiple comparisons. Analyses were performed using Statistica 13 PL and R 4.6.1.

Results

Twenty-five patients with pemphigus vulgaris or pemphigoid were identified as potentially eligible for this analysis (Fig. 1). Two were excluded based on the clinical exclusion criteria: one because of active recurrent oral candidiasis and one patient with mucous membrane pemphigoid because of methotrexate treatment for rheumatoid arthritis. The final cohort comprised 23 patients: 12 with pemphigus vulgaris and 11 with pemphigoid (10 with mucous membrane pemphigoid and one with bullous pemphigoid). All 23 patients were included retrospectively. Three participants were receiving topical glucocorticosteroids at imaging (two with pemphigus vulgaris and one with pemphigoid).

Baseline characteristics are shown in Table 1. Median age was 47.0 years in pemphigus vulgaris and 69.0 years in pemphigoid. Women represented 66.7% and 90.9%, respectively. Median symptom duration before dermatological consultation was 4.0 and 8.0 months, while median pain VAS was 8.0 and 7.0, and oral ODSS was 36.5 and 25.0. All pemphigus vulgaris patients and one patient with pemphigoid had a positive Nikolsky sign. Erythema was present in all patients. Erosions occurred in all patients with pemphigus vulgaris and in 9/11 patients with pemphigoid, whereas visible blisters were observed only in patients with pemphigoid (3/11).

Table 1
Demographic and clinical characteristics

Characteristic	PV, n = 12	Pemphigoid, n = 11
Demographic characteristics		
Age, years	47.0 (42.0-50.5)	69.0 (61.0–82.0)
Female sex	8/12 (66.7)	10/11 (90.9)
Duration of symptoms before dermatology consultation, months	4.0 (2.5–12.0)	8.0 (6.5–18.0)
Clinical severity		
Pain VAS score	8.0 (7.0-9.5)	7.0 (6.0–8.0)
Oral mucosal ODSS score	36.5 (27.0-50.5)	25.0 (15.0–29.0)
PDAI score	15.0 (10.5–25.0)	-
MMPDAI score	-	25.0 (20.0–28.0)
Clinical findings		
Positive Nikolsky sign	12/12 (100.0)	1/11 (9.1)
Erythema present	12/12 (100.0)	11/11 (100.0)
Blisters present	0/12 (0.0)	3/11 (27.3)
Erosions present	12/12 (100.0)	9/11 (81.8)

Values are median (IQR) or n/N (%). Pemphigoid comprised 10 patients with MMP and one with BP. PDAI was assessed only in PV; MMPDAI was used descriptively as a mucosal activity measure in pemphigoid. **Abbreviations**: BP, bullous pemphigoid; IQR, interquartile range; MMP, mucous membrane pemphigoid; MMPDAI, Mucous Membrane Pemphigoid Disease Area Index; ODSS, Oral Disease Severity Score; PDAI, Pemphigus Disease Area Index; PV, pemphigus vulgaris; VAS, visual analogue scale.

Gingival involvement was extensive in both diseases (Table 2). The upper attached gingiva was more frequently involved in patients with pemphigoid than in those with pemphigus vulgaris (100.0% vs 58.3%; p = 0.037). Other gingival comparisons were not nominally significant. In both diseases, the buccal mucosa was the most frequently affected non-gingival oral site (pemphigus vulgaris, 83.3%; pemphigoid, 54.5%; Table 2). Labial mucosal involvement (58.3% vs 0%; p = 0.005) and genital mucosal involvement (41.7% vs 0%; p = 0.037) were more frequent in pemphigus vulgaris. Tongue and scalp involvement occurred only in pemphigus vulgaris, whereas hard palate involvement occurred only in pemphigoid, but these differences were not nominally significant.

Table 2
Clinical distribution of lesions **Gingival involvement**

Site	PV, n/N (%)	Pemphigoid, n/N (%)	PV-pemphigoid difference, pp (95% CI)	p-value
Upper marginal gingiva	7/12 (58.3)	10/11 (90.9)	-32.6 (-65.2 to + 0.1)	0.155
Upper attached gingiva	7/12 (58.3)	11/11 (100.0)	-41.7 (-69.6 to -13.8)	0.037
Upper alveolar gingiva	10/12 (83.3)	10/11 (90.9)	-7.6 (-34.7 to + 19.5)	1.000
Lower marginal gingiva	11/12 (91.7)	10/11 (90.9)	+ 0.8 (-22.3 to + 23.8)	1.000
Lower attached gingiva	11/12 (91.7)	11/11 (100.0)	-8.3 (-24.0 to + 7.3)	1.000
Lower alveolar gingiva	10/12 (83.3)	11/11 (100.0)	-16.7 (-37.8 to + 4.4)	0.478

Non-gingival involvement

Site	PV, n/N (%)	Pemphigoid, n/N (%)	PV-pemphigoid difference, pp (95% CI)	p-value
Buccal mucosa	10/12 (83.3)	6/11 (54.5)	+ 28.8 (-7.4 to + 65.0)	0.193
Tongue	4/12 (33.3)	0/11 (0.0)	+ 33.3 (+ 6.7 to + 60.0)	0.093
Hard palate	0/12 (0.0)	3/11 (27.3)	-27.3 (-53.6 to -1.0)	0.093
Soft palate	3/12 (25.0)	1/11 (9.1)	+ 15.9 (-13.9 to + 45.7)	0.590
Floor of the mouth	2/12 (16.7)	0/11 (0.0)	+ 16.7 (-4.4 to + 37.8)	0.478
Labial mucosa	7/12 (58.3)	0/11 (0.0)	+ 58.3 (+ 30.4 to + 86.2)	0.005
Vermilion	4/12 (33.3)	1/11 (9.1)	+ 24.2 (-7.4 to + 55.9)	0.317
Non-hair-bearing skin	5/12 (41.7)	2/11 (18.2)	+ 23.5 (-12.5 to + 59.5)	0.371
Scalp	4/12 (33.3)	0/11 (0.0)	+ 33.3 (+ 6.7 to + 60.0)	0.093
Genital mucosa	5/12 (41.7)	0/11 (0.0)	+ 41.7 (+ 13.8 to + 69.6)	0.037

Values are n/N (%). Differences are PV-pemphigoid percentage-point differences with unpooled Wald 95% CIs. P-values are from two-sided Fisher exact tests and are exploratory (no multiplicity adjustment).
Abbreviations: CI, confidence interval; PV, pemphigus vulgaris; pp, percentage points.

LC-OCT findings

All LC-OCT examinations were evaluable. Pemphigus vulgaris was characterized by partial-thickness epithelial disruption and by hyperreflective, gray, drop-shaped elements attached to the cleft roof and floating within the cleft (Fig. 2 and Supplementary Figs. 1–3). Both features were observed in all participants with pemphigus vulgaris and in none with pemphigoid (12/12 vs 0/11; both FDR-adjusted p-values < 0.001). Hyporeflective intraepithelial clefts were identified in 8/12 participants with pemphigus vulgaris and none with pemphigoid (0/11; FDR-adjusted p = 0.004) (Fig. 2 and Supplementary Figs. 1–4). Conversely, a hyporeflective subepithelial cleft containing homogeneous, mildly reflective material and small bright particles was observed in all participants with pemphigoid and none with pemphigus vulgaris (11/11 vs 0/12; FDR-adjusted p < 0.001). Among those with pemphigoid, subepithelial clefts showed two morphological patterns: unilocular in 7/11 and multilocular in 4/11 (Table 3; Fig. 3, Supplementary Figs. 5,6).

Table 3
LC-OCT features

LC-OCT feature	PV, n/N (%)	Pemphigoid, n/N (%)	PV-pemphigoid difference, pp (95% CI)	Raw p / FDR-adjusted p	Interobserver agreement, %
Epithelial continuity: partial-thickness disruption (erosion)	12/12 (100.0)	0/11 (0.0)	+ 100.0 (+ 100.0 to + 100.0)	< 0.001 / <0.001	100.0
Epithelial continuity: full-thickness disruption	0/12 (0.0)	2/11 (18.2)	-18.2 (-41.0 to + 4.6)	0.217 / 0.318	100.0
Parakeratosis	0/12 (0.0)	1/11 (9.1)	-9.1 (-26.1 to + 7.9)	0.478 / 0.535	100.0
Hyperkeratosis	1/12 (8.3)	3/11 (27.3)	-18.9 (-49.6 to + 11.7)	0.317 / 0.430	100.0
Disrupted epithelial architecture	11/12 (91.7)	6/11 (54.5)	+ 37.1 (+ 3.8 to + 70.4)	0.069 / 0.130	87.0
Small bright particles (inflammatory cells) in the epithelium	11/12 (91.7)	5/11 (45.5)	+ 46.2 (+ 12.9 to + 79.5)	0.027 / 0.065	95.7
Altered basement membrane zone	11/12 (91.7)	11/11 (100.0)	-8.3 (-24.0 to + 7.3)	1.000 / 1.000	100.0
Small bright particles (inflammatory cells) in the basement membrane	0/12 (0.0)	11/11 (100.0)	-100.0 (-100.0 to -100.0)	< 0.001 / <0.001	100.0
Small bright particles (inflammatory cells) in the lamina propria	2/12 (16.7)	4/11 (36.4)	-19.7 (-55.1 to + 15.7)	0.371 / 0.470	95.7
Villous formation	2/12 (16.7)	0/11 (0.0)	+ 16.7 (-4.4 to + 37.8)	0.478 / 0.535	100.0
Hyperreflective, gray, drop-shaped elements attached to the cleft roof and floating within the cleft (acantholytic cells)	12/12 (100.0)	0/11 (0.0)	+ 100.0 (+ 100.0 to + 100.0)	< 0.001 / <0.001	100.0
Hyporeflective intraepithelial cleft (intraepithelial blister)	8/12 (66.7)	0/11 (0.0)	+ 66.7 (+ 40.0 to + 93.3)	0.001 / 0.004	100.0
Hyporeflective subepithelial cleft containing homogeneous, mildly reflective material and	0/12 (0.0) 0/12 (0.0)	11/11 (100.0) 7/11 (63.6)	-100.0 (-100.0 to -100.0)	< 0.001 / <0.001 0.001 / 0.004	100.0 100.0 100.0

LC-OCT feature	PV, n/N (%)	Pemphigoid, n/N (%)	PV-pemphigoid difference, pp (95% CI)	Raw p / FDR-adjusted p	Interobserver agreement, %
small bright particles (subepithelial blister filled with fibrin and inflammatory cells) Subtypes: Unilocular Multilocular	0/12 (0.0)	4/11 (36.4)	-63.6 (-92.1 to -35.2) -36.4 (-64.8 to -7.9)	0.037 / 0.079	
Blister content: moderately reflective, roundish floating elements (inflammatory cells and erythrocytes)	2/12 (16.7)	9/11 (81.8)	-65.2 (-96.2 to -34.1)	0.003 / 0.009	100.0
Festooning of connective tissue papillae at the base of the blister	0/12 (0.0)	2/11 (18.2)	-18.2 (-41.0 to + 4.6)	0.217 / 0.318	95.7
Vessels visible	10/12 (83.3)	8/11 (72.7)	+ 10.6 (-23.1 to + 44.3)	0.640 / 0.676	100.0
Vessels dilated	5/10 (50.0)	1/8 (12.5)	+ 37.5 (-1.0 to + 76.0)	0.152 / 0.262	100.0

Values are n/N (%). Differences are PV-pemphigoid percentage-point differences with unpooled Wald 95% CIs. Raw p-values are from two-sided Fisher exact tests; FDR-adjusted p-values use the Benjamini-Hochberg procedure across 19 comparisons and were calculated from unrounded raw p-values. Interobserver agreement is the percentage of concordant paired ratings before consensus review. For vessel dilation, denominators include only participants with visible vessels (PV, n = 10; pemphigoid, n = 8). **Abbreviations**: CI, confidence interval; FDR, false discovery rate; LC-OCT, line-field confocal optical coherence tomography; PV, pemphigus vulgaris; pp, percentage points.

Moderately reflective, round, floating elements within blisters were more frequent in patients with pemphigoid than in those with pemphigus vulgaris (81.8% vs 16.7%; FDR-adjusted p = 0.009). Epithelial small bright particles were more frequent in pemphigus vulgaris (91.7% vs 45.5%; raw p = 0.027); however, this difference was not statistically significant after FDR correction (adjusted p = 0.065). No other epithelial, lamina propria, and vascular LC-OCT features differed significantly.

Interobserver agreement and correlation analyses

Feature-specific interobserver agreement ranged from 87.0% to 100.0% and was 100% for partial-thickness epithelial disruption, hyperreflective, gray, drop-shaped elements, and intraepithelial and

subepithelial clefts (Table 3). In pemphigus vulgaris, no statistically significant correlations were observed between epithelial small bright particles and DIF intensity, ODSS, PDAI, pemphigus IIF titer, anti-Dsg1 or anti-Dsg3 antibody levels, or symptom duration (Supplementary Table 1). The strongest correlation was observed with anti-Dsg3 antibody levels (rho = 0.53; p = 0.074). Correlations could not be calculated in patients with pemphigoid because small bright particles in BMZ showed no variability (11/11 present).

Videodermoscopic findings

Curved vessels were more frequent in patients with pemphigoid than in those with pemphigus vulgaris (72.7% vs 16.7%; p = 0.012; Supplementary Table 2). Other vessel patterns did not differ.

Discussion

Pemphigus vulgaris and pemphigoid showed distinct LC-OCT patterns reflecting their different levels of tissue separation. Pemphigus vulgaris was characterized by partial-thickness epithelial disruption consistent with erosion, hyperreflective, gray, drop-shaped elements attached to the cleft roof and floating within the cleft, interpreted as acantholytic cells, and hyporeflective intraepithelial clefts. In contrast, pemphigoid was characterized by a hyporeflective subepithelial cleft containing homogeneous, mildly reflective material and small bright particles, compatible with a subepithelial blister containing fibrin and inflammatory cells together with small bright particles reflective of inflammatory cells in the basement membrane. These findings were interpreted in the context of LC-OCT features of cutaneous autoimmune bullous diseases described by Tognetti et al. [10] and Gellrich et al. [12], as well as OCT and reflectance confocal microscopy (RCM) findings reported by Mogensen et al. [20] and Ardigò et al. [21].

LC-OCT features of the epithelium

LC-OCT features interpreted as acantholytic cells were observed exclusively in pemphigus vulgaris, supporting their value as a distinguishing LC-OCT feature. Tognetti et al. [10] reported a similar pattern in cutaneous pemphigus, with large (12–20 μm), roundish, hyperreflective structures. The presence of similar structures in gingival lesions may represent an in vivo correlate of keratinocyte detachment following loss of intercellular adhesion. This interpretation is consistent with the histopathological mechanism described by Lanza et al. [22] and previous RCM and OCT observations reported by Alessi et al. [1] and Panzarella et al. [23, 24]. Although intact intraepithelial blisters were also more frequent in pemphigus vulgaris than in pemphigoid, they were observed less often than acantholytic cells, because fragile oral blisters rupture rapidly and leave erosions visible as partial-thickness epithelial disruption.

Villous formation was more frequent in pemphigus vulgaris, although the difference was not statistically significant, and may represent an LC-OCT correlate of papillary projections of the lamina propria lined by preserved basal epithelium after suprabasal acantholysis. This interpretation is supported by Alessi et al.

[1], who described preservation of the basal epithelial layer, with a clearly visible keratinocyte rim around the papillae in pemphigus vulgaris.

Intraepithelial small bright particles were also more frequent in pemphigus vulgaris than in pemphigoid, but this difference did not remain significant after FDR correction. Alessi et al. [1] described similar bright structures within the epithelial honeycomb pattern in pemphigus vulgaris and interpreted them as inflammatory cells.

The attached gingiva is masticatory mucosa covered by keratinized or parakeratinized stratified squamous epithelium and adapted to mechanical function, as described by Dawson et al. [25]. Therefore, focal hyperkeratosis or parakeratosis, which did not differentiate pemphigus vulgaris from pemphigoid, may reflect reactive keratinization associated with chronic epithelial injury and local friction rather than disease-specific LC-OCT features, consistent with observations by Mignogna et al. [26] and Müller [27].

In pemphigus vulgaris, intraepithelial small bright particles showed no statistically significant correlations with mucosal ODSS, PDAI, IIF IgG pemphigus level, ELISA anti-Dsg1 level, or disease duration. The correlation estimate for ELISA anti-Dsg3 level was moderate and positive, which is noteworthy because Weiss et al. [28] reported that anti-Dsg3 levels may reflect disease activity in pemphigus vulgaris. However, the estimate did not meet the conventional threshold for statistical significance and should be regarded as a potential signal requiring evaluation in a larger study.

Intraepithelial small bright particles also showed no statistically significant correlations with DIF IgG, IgA or C3 intensity in pemphigus vulgaris, providing no evidence in this study that they track immune-deposit intensity.

LC-OCT features of the BMZ and subepithelial compartment

On LC-OCT, altered BMZ was common in both pemphigus vulgaris and pemphigoid, but the associated findings differed. In pemphigoid, it was accompanied by small bright particles at the junction between the epithelium and connective tissue. These particles were present in all participants with pemphigoid and absent in those with pemphigus vulgaris. They may represent inflammatory cells and contribute to the altered appearance of the BMZ. In pemphigus vulgaris, altered BMZ may instead result from suprabasal acantholysis and intraepithelial blister formation, which disrupt the optical appearance of the junction rather than indicate primary BMZ involvement.

Correlations between BMZ small bright particles and DIF intensity, clinical activity scales, or circulating anti-BMZ antibodies could not be calculated because these particles were present in all patients with pemphigoid and therefore showed no variability. Rashid et al. [4] also noted that circulating BMZ autoantibodies are often absent or detectable only at low titres by standard IIF, which may further limit correlations with serological measures in small samples of patients with mucous membrane pemphigoid.

The subepithelial location of the blisters was the principal LC-OCT feature distinguishing between pemphigus vulgaris and pemphigoid and reflected the expected level of tissue separation. Both unilocular and multilocular patterns were observed, complementing the OCT findings of Panzarella et al. [24], who described multilocular, subepithelial blisters as characteristic of mucous membrane pemphigoid. The homogeneous, mildly reflective material may represent fibrin, whereas the moderately reflective, roundish, floating elements likely represent cellular components. Because LC-OCT did not allow reliable differentiation between inflammatory cells and erythrocytes, these elements could represent either cell type or both. This interpretation is consistent with Tognetti et al. [10], who described fibrin bundles, erythrocytes, and inflammatory cells within cutaneous mucous membrane pemphigoid blisters, and with Ardigò et al. [21], who correlated mildly reflective homogeneous material in pemphigoid lesions with fibrin deposition.

Small bright particles in the lamina propria interpreted as inflammatory cells did not clearly differ between pemphigus vulgaris and pemphigoid. As Laurell et al. [29] showed that inflammatory cells may also be present in clinically healthy gingival connective tissue, this finding should be regarded as a nonspecific inflammatory feature.

Festooning of the connective tissue papillae was rarely observed and did not distinguish pemphigus vulgaris from pemphigoid in our cohort. Histopathologically, festooning describes preserved papillary projections extending into the blister cavity, as reported by Calonje et al. [30] in subepidermal blistering disorders.

LC-OCT and videodermoscopic features of blood vessels

Vessel dilation was more frequent in pemphigus vulgaris, but the difference was not statistically significant. Alessi et al. [1] described dilated vessels in both pemphigus vulgaris and mucous membrane pemphigoid gingival lesions using RCM. Kurzeja et al. [31] reported multiple dilated blood vessels in cutaneous pemphigus vulgaris. These observations support the occurrence of vessel dilation in pemphigus but not its specificity for differentiating pemphigus vulgaris from pemphigoid. On videodermoscopy, curved vessels were nominally more frequent in pemphigoid than in pemphigus vulgaris, whereas looped, serpentine, coiled, and arborizing vessels did not differ significantly.

Clinical distribution of lesions

The present study assessed the marginal, attached, and alveolar gingiva of the maxilla and mandible separately.

In pemphigus vulgaris, gingival involvement was substantial but not uniform. The lower marginal and attached gingiva were most frequently affected, followed by the lower and upper alveolar gingiva. Direct comparison with previous studies is limited because Scully et al. [32], Sultan et al. [2], and Mignogna et al. [33] generally reported desquamative gingivitis as a single clinical category rather than by specific gingival region.

The buccal mucosa was the most frequently affected non-gingival oral site in pemphigus vulgaris, involving 10/12 patients (83.3%), comparable to the 90% reported by Fernández et al. [34]. Other sites included the labial mucosa (58.3%), tongue and vermilion (33.3% each), soft palate (25.0%), and floor of the mouth (16.7%), supporting a disseminated pattern of oral involvement. These sites appeared more frequently involved than in the study by Sultan et al. [2].

Extraoral involvement was also notable in pemphigus vulgaris: non-hair-bearing skin and genital mucosa were each affected in 41.7% of patients, and the scalp in 33.3%. Cutaneous involvement was more frequent than the 24% reported by Scully et al. [32]. Genital involvement was similar to that reported by Kavala et al. [35] (44.1%) and Akhyani et al. [36] (51%). Scalp lesions were less frequent than in the study by Sar-Pomian et al. [37] (65.2%). Together, these findings support the diffuse mucocutaneous distribution of pemphigus vulgaris.

In all patients with pemphigoid, extensive gingival involvement was observed (Table 2), consistent with the findings of Sultan et al. [2] and Bagan et al. [38]. Among patients with pemphigoid, the buccal mucosa was the most frequently affected non-gingival oral site, with lesions in 6/11 patients (54.5%). This is comparable to the 58% reported by Sultan et al. [2]. Hard and soft palate involvement occurred in a minority of patients. No involvement of the tongue, floor of the mouth, or labial mucosa was observed, consistent with reports by Sultan et al. [2] and Bagan et al. [38]. Extraoral involvement in pemphigoid was limited.

Limitations

The small sample from a single-center study limited the precision of the findings. Histopathological examination was not performed at the LC-OCT imaging sites, preventing direct correlation between imaging and tissue findings. The inclusion of one patient with bullous pemphigoid introduced limited clinical heterogeneity. Therefore, the findings should be interpreted as applying to the pemphigoid group and not as specific to mucous membrane pemphigoid, although the principal subepithelial LC-OCT pattern was present in all 11 patients. The generalizability of these LC-OCT patterns to disease stages or imaging protocols requires confirmation in multicenter cohorts.

Conclusions

Line-field confocal optical coherence tomography may serve as an adjunctive method by providing useful real-time, noninvasive information that supports differentiation between pemphigus vulgaris and pemphigoid presenting as desquamative gingivitis. The characteristic pemphigus vulgaris pattern included features interpreted as acantholytic cells, partial-thickness epithelial disruption, and intraepithelial clefts, whereas pemphigoid was characterized by subepithelial clefts containing homogeneous, mildly reflective material and small bright particles. Pemphigus vulgaris showed more extensive mucocutaneous involvement, whereas pemphigoid predominantly affected the gingiva, with the buccal mucosa as the most common non-gingival oral site. This difference in lesion distribution may

provide complementary clinical clues. Prospective longitudinal studies should determine whether LC-OCT can also be used to monitor disease activity and treatment response.

Declarations

Compliance with Ethical Standards

Acknowledgments:

Ethics approval

statement: This study was performed in accordance with the principles of the Declaration of Helsinki. The study protocol for the retrospective use of existing clinical records and archived LC-OCT images was approved by the Bioethics Committee of the Medical University of Warsaw on 4 May 2026 (approval no. KB/28/2026). Data generated before that date had been obtained during routine clinical care and were accessed and analyzed for research purposes only after ethical approval had been granted.

Informed consent

statement: Written informed consent for participation and the scientific use of clinical and imaging data was obtained from all participants.

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Conflicts of interest: The authors declare no conflicts of interest.

Author Contribution

U.F. and M.O. drafted the main manuscript text. U.F. and M.K. independently assessed the LC-OCT images. U.F., C.S., M.K., A.P., J.C., L.R., and M.O. contributed to the study design, data acquisition, analysis, or interpretation of the findings. All authors critically revised the manuscript, approved the final version, and agree to be accountable for all aspects of the work.

Data Availability

The identified individual, clinical and LC-OCT imaging data supporting the findings of this study are not publicly available because they contain sensitive patient information. They may be made available by the corresponding author upon reasonable request, subject to ethics approval and applicable data protection

restrictions. The images included in the article and its electronic supplementary material are available with the publication.

References

1. Alessi SS, Nico MMS, Fernandes JD, Lourenço SV (2013) Reflectance confocal microscopy as a new tool in the in vivo evaluation of desquamative gingivitis: patterns in mucous membrane pemphigoid, pemphigus vulgaris and oral lichen planus. Br J Dermatol 168:257–264. https://doi.org/10.1111/bjd.12021
2. Sultan AS, Villa A, Saavedra AP, Treister NS, Woo SB (2017) Oral mucous membrane pemphigoid and pemphigus vulgaris - a retrospective two-center cohort study. Oral Dis 23:498–504. https://doi.org/10.1111/odi.12639
3. Joly P, Horvath B, Patsatsi A, Uzun S, Bech R, Beissert S et al (2020) Updated S2K guidelines on the management of pemphigus vulgaris and foliaceus initiated by the European Academy of Dermatology and Venereology (EADV). J Eur Acad Dermatol Venereol 34:1900–1913. https://doi.org/10.1111/jdv.16752
4. Rashid H, Lamberts A, Borradori L, Alberti-Violetti S, Barry RJ, Caproni M et al (2021) European guidelines (S3) on diagnosis and management of mucous membrane pemphigoid, initiated by the European Academy of Dermatology and Venereology - Part I. J Eur Acad Dermatol Venereol 35:1750–1764. https://doi.org/10.1111/jdv.17397
5. Carey B, Joshi S, Abdelghani A, Mee J, Andiappan M, Setterfield J (2020) The optimal oral biopsy site for diagnosis of mucous membrane pemphigoid and pemphigus vulgaris. Br J Dermatol 182:747–753. https://doi.org/10.1111/bjd.18032
6. Daltaban Ö, Özçentik A, Akman Karakaş A, Üstün K, Hatipoğlu M, Uzun S (2020) Clinical presentation and diagnostic delay in pemphigus vulgaris: a prospective study from Turkey. J Oral Pathol Med 49:681–686. https://doi.org/10.1111/jop.13052
7. Caggiula A, Romano F, Dentico E, Pinto F, Lopez-Jornet MP, Petruzzi M (2026) Diagnostic delay in desquamative gingivitis: an observational cohort study. Oral Dis Adv online publication. https://doi.org/10.1111/odi.70276
8. Capocasale G, Panzarella V, Rodolico V, Di Fede O, Campisi G (2018) In vivo optical coherence tomography imaging in a case of mucous membrane pemphigoid and a negative Nikolsky's sign. J Dermatol 45:603–605. https://doi.org/10.1111/1346-8138.14267
9. Obade AY, Pandarathodiyil AK, Oo AL, Warnakulasuriya S, Ramanathan A (2021) Application of optical coherence tomography to study the structural features of oral mucosa in biopsy tissues of oral dysplasia and carcinomas. Clin Oral Investig 25:5411–5419. https://doi.org/10.1007/s00784-021-03849-0
10. Tognetti L, Cinotti E, Suppa M, Guazzo R, Habougit C, Santi F et al (2021) Line field confocal optical coherence tomography: an adjunctive tool in the diagnosis of autoimmune bullous diseases. J Biophotonics 14:e202000449. https://doi.org/10.1002/jbio.202000449

11. Fałkowska U, Durmaj A, Szwed C, Kurzeja M, Czuwara J, Olczak-Kowalczyk D et al (2026) Noninvasive imaging techniques as modern diagnostic tools in desquamative gingivitis: focus on RCM, OCT, and LC-OCT. Skin Res Technol 32:e70236. https://doi.org/10.1111/srt.70236
12. Gellrich FF, Rakhmetova S, Günther C, Beissert S, Hobelsberger S (2025) Real-time, noninvasive diagnosis of bullous pemphigoid: diagnostic performance of line-field confocal optical coherence tomography. Skin Res Technol 31:e70235. https://doi.org/10.1111/srt.70235
13. Zhao L, Chen Y, Wang M (2023) The global incidence rate of pemphigus vulgaris: a systematic review and meta-analysis. Dermatology 239:514–522. https://doi.org/10.1159/000530121
14. Du G, Patzelt S, van Beek N, Schmidt E (2022) Mucous membrane pemphigoid. Autoimmun Rev 21:103036. https://doi.org/10.1016/j.autrev.2022.103036
15. Ormond M, McParland H, Donaldson ANA, Andiappan M, Cook RJ, Escudier M et al (2018) An Oral Disease Severity Score validated for use in oral pemphigus vulgaris. Br J Dermatol 179:872–881. https://doi.org/10.1111/bjd.16265
16. Ormond M, McParland H, Thakrar P, Donaldson ANA, Andiappan M, Cook RJ et al (2020) Validation of an Oral Disease Severity Score (ODSS) tool for use in oral mucous membrane pemphigoid. Br J Dermatol 183:78–85. https://doi.org/10.1111/bjd.18566
17. Rosenbach M, Murrell DF, Bystryn JC, Dulay S, Dick S, Fakharzadeh S et al (2009) Reliability and convergent validity of two outcome instruments for pemphigus. J Invest Dermatol 129:2404–2410. https://doi.org/10.1038/jid.2009.72
18. Murrell DF, Marinovic B, Caux F, Prost C, Ahmed R, Wozniak K et al (2015) Definitions and outcome measures for mucous membrane pemphigoid: recommendations of an international panel of experts. J Am Acad Dermatol 72:168–174. https://doi.org/10.1016/j.jaad.2014.08.024
19. Oetter N, Pröll J, Sievert M, Goncalves M, Rohde M, Nobis CP et al (2024) Oral mucosa - an examination map for confocal laser endomicroscopy within the oral cavity: an experimental clinical study. Clin Oral Investig 28:266. https://doi.org/10.1007/s00784-024-05664-9
20. Mogensen M, Morsy HA, Nürnberg BM, Jemec GBE (2008) Optical coherence tomography imaging of bullous diseases. J Eur Acad Dermatol Venereol 22:1458–1464. https://doi.org/10.1111/j.1468-3083.2008.02917.x
21. Ardigò M, Agozzino M, Amorosi B, Moscarella E, Cota C, De Abreu L et al (2014) Real-time, non-invasive microscopic confirmation of clinical diagnosis of bullous pemphigoid using in vivo reflectance confocal microscopy. Skin Res Technol 20:194–199. https://doi.org/10.1111/srt.12105
22. Lanza A, Cirillo N, Femiano F, Gombos F (2006) How does acantholysis occur in pemphigus vulgaris: a critical review. J Cutan Pathol 33:401–412. https://doi.org/10.1111/j.0303-6987.2006.00523.x
23. Panzarella V, Bartolone A, Rodolico V, Capocasale G, Maniscalco L, Matranga D et al (2021) Immune-mediated desquamative gingivitis and optical coherence tomography diagnostic patterns: clinical implication from a systematic review. Diagnostics (Basel) 11:1453. https://doi.org/10.3390/diagnostics11081453

24. Panzarella V, Bartolone A, Coniglio R, Rodolico V, Maniscalco L, Capocasale G et al (2021) Diagnostic concordance between optical coherence tomography and histological investigations for immune-mediated desquamative gingivitis: observational study. Int J Environ Res Public Health 18:9095. https://doi.org/10.3390/ijerph18179095
25. Dawson DV, Drake DR, Hill JR, Brogden KA, Fischer CL, Wertz PW (2013) Organization, barrier function and antimicrobial lipids of the oral mucosa. Int J Cosmet Sci 35:220–223. https://doi.org/10.1111/ics.12038
26. Mignogna MD, Fortuna G, Leuci S, Adamo D, Siano M, Makary C et al (2011) Frictional keratoses on the facial attached gingiva are rare clinical findings and do not belong to the category of leukoplakia. J Oral Maxillofac Surg 69:1367–1374. https://doi.org/10.1016/j.joms.2010.05.087
27. Müller S (2019) Frictional keratosis, contact keratosis and smokeless tobacco keratosis: features of reactive white lesions of the oral mucosa. Head Neck Pathol 13:16–24. https://doi.org/10.1007/s12105-018-0986-3
28. Weiss D, Ristl R, Griss J, Bangert C, Foedinger D, Stingl G et al (2015) Autoantibody levels and clinical disease severity in patients with pemphigus: comparison of aggregated anti-desmoglein ELISA values and indirect immunofluorescence titres. Acta Derm Venereol 95:559–564. https://doi.org/10.2340/00015555-2023
29. Laurell L, Rylander H, Sundin Y (1987) Histologic characteristics of clinically healthy gingiva in adolescents. Scand J Dent Res 95:456–462. https://doi.org/10.1111/j.1600-0722.1987.tb01960.x
30. Calonje E, Brenn T, Lazar A, McKee PH (2012) McKee's pathology of the skin: with clinical correlations, 4th edn. W.B. Saunders Company, Philadelphia
31. Kurzeja M, Rakowska A, Rudnicka L, Olszewska M (2012) Criteria for diagnosing pemphigus vulgaris and pemphigus foliaceus by reflectance confocal microscopy. Skin Res Technol 18:339–346. https://doi.org/10.1111/j.1600-0846.2011.00574.x
32. Scully C, De Paes O, Porter SR, Gilkes JJ (1999) Pemphigus vulgaris: the manifestations and long-term management of 55 patients with oral lesions. Br J Dermatol 140:84–89. https://doi.org/10.1046/j.1365-2133.1999.02612.x
33. Mignogna MD, Lo Muzio L, Bucci E (2001) Clinical features of gingival pemphigus vulgaris. J Clin Periodontol 28:489–493. https://doi.org/10.1034/j.1600-051x.2001.028005489.x
34. Fernández S, España A, Navedo M, Barona L (2012) Study of oral, ear, nose and throat involvement in pemphigus vulgaris by endoscopic examination. Br J Dermatol 167:1011–1016. https://doi.org/10.1111/j.1365-2133.2012.11098.x
35. Kavala M, Topaloğlu Demir F, Zindanci I, Can B, Turkoğlu Z, Zemheri E et al (2015) Genital involvement in pemphigus vulgaris: correlation with clinical and cervicovaginal Pap smear findings. J Am Acad Dermatol 73:655–659. https://doi.org/10.1016/j.jaad.2015.06.057
36. Akhyani M, Chams-Davatchi C, Naraghi Z, Daneshpazhooh M, Toosi S, Asgari M et al (2008) Cervicovaginal involvement in pemphigus vulgaris: a clinical study of 77 cases. Br J Dermatol 158:478–482. https://doi.org/10.1111/j.1365-2133.2007.08356.x

37. Sar-Pomian M, Konop M, Gala K, Rudnicka L, Olszewska M (2018) Scalp involvement in pemphigus: a prognostic marker. Postepy Dermatol Alergol 35:293–298. https://doi.org/10.5114/pdia.2017.71267
38. Bagan J, Jiménez Y, Murillo J, Bagan L (2018) Oral mucous membrane pemphigoid: a clinical study of 100 low-risk cases. Oral Dis 24:132–134. https://doi.org/10.1111/odi.12744

Figures

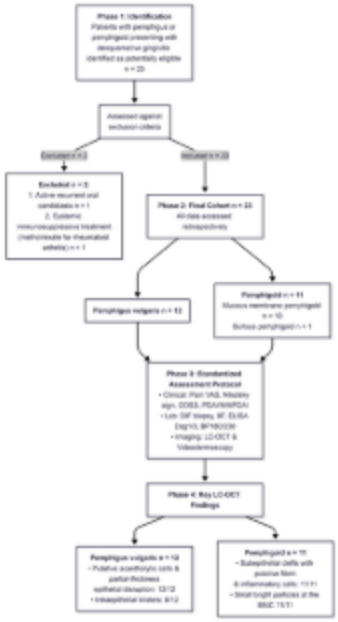


Figure 1

Patient flow diagram. Twenty-five patients with an immunologically confirmed diagnosis of pemphigus vulgaris or pemphigoid were identified as potentially eligible; two were excluded (active recurrent oral candidiasis, n=1; methotrexate treatment for rheumatoid arthritis, n=1). The final cohort comprised 23 patients: 12 with pemphigus vulgaris and 11 with pemphigoid (10 with mucous membrane pemphigoid and 1 with bullous pemphigoid); all 23 patients were included retrospectively. Following a standardized assessment protocol, LC-OCT imaging revealed distinct differentiating patterns: putative acantholytic cells and intraepithelial clefts in pemphigus vulgaris, and subepithelial clefts in pemphigoid.

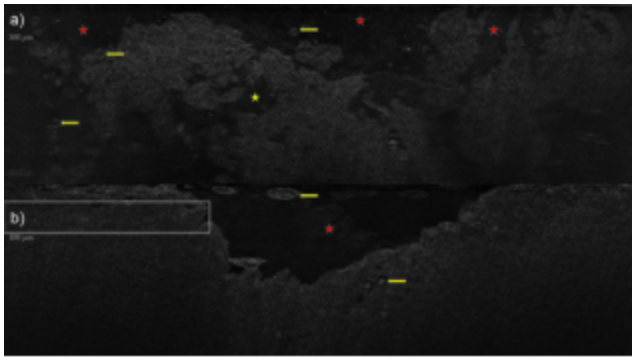


Figure 2

Pemphigus vulgaris. LC-OCT image in the vertical plane. Scale bar = 100 µm. Yellow arrows indicate hyperreflective, gray, drop-shaped elements interpreted as acantholytic cells; red asterisks indicate partial-thickness disruptions of epithelial continuity; the yellow asterisk indicates an intraepithelial blister

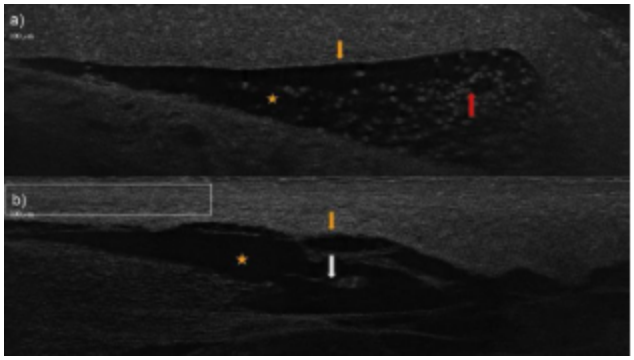


Figure 3

Pemphigoid. LC-OCT image in the vertical plane. Scale bar = 100 µm. Orange asterisks indicate a subepithelial unilocular blister; red arrows indicate blister content consisting of moderately reflective, roundish, floating elements compatible with inflammatory cells and erythrocytes; gray arrows indicate homogeneous, mildly reflective material and hyporeflective elongated structures compatible with fibrin deposition; orange arrows indicate an altered basement membrane zone with small bright particles interpreted as inflammatory cells

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