

Evaluation of the Diagnostic Value of Oesophageal Biopsies for Direct Immunofluorescence Microscopy in Mucous Membrane Pemphigoid

Kaan YILMAZ^{1,2}, Onur DIKMEN^{1,3}, Nina VAN BEEK¹, Jens U. MARQUARDT⁴, Martha M. KIRSTEIN⁴, Detlef ZILLIKENS¹ and Enno SCHMIDT^{1,5}

¹Department of Dermatology, University of Lübeck, Lübeck, ²Department of Dermatology, University of Heidelberg, Medical Faculty Mannheim, Mannheim, ³Charité – Universitätsmedizin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Pathology, Berlin, ⁴Department of Medicine I, University of Lübeck, Lübeck and ⁵Lübeck Institute of Experimental Dermatology (LIED), University of Lübeck, Lübeck, Germany

Mucous membrane pemphigoid is an autoimmune blistering disorder characterized by predominant involvement of surface-close epithelia and linear depositions of immunoreactants at the dermal-epithelial junction on direct immunofluorescence microscopy. A major diagnostic difficulty is the frequent need for multiple biopsies to facilitate the diagnosis. Although oesophageal involvement is a rare, but life-threatening manifestation, the relevance of oesophageal direct immunofluorescence sampling is unclear. This retrospective monocentric study evaluated 67 non-lesional biopsies from 11 patients with mucous membrane pemphigoid and clinical symptoms suggestive of oesophageal involvement, comprising 31 samples from the oesophagus and 36 samples from other anatomical sites. Five patients (45.5%) exhibited endoscopic findings compatible with oesophageal involvement of mucous membrane pemphigoid. No correlation was identified between the presence of oesophageal lesions and direct immunofluorescence positivity in lesions from the oesophagus ($p = 1.0$). Oral and cutaneous samples were significantly more frequently positive by direct immunofluorescence than were oesophageal biopsies ($p < 0.0001$ and $p = 0.0195$, respectively). Oesophageal samples yielded significantly less IgG reactivity than oral and cutaneous lesions ($p < 0.0001$ and $p = 0.0126$, respectively), and less IgA antibody response than oral lesions ($p = 0.0036$). In conclusion, oesophageal direct immunofluorescence samples were inferior to oral and cutaneous biopsies for the diagnosis of mucous membrane pemphigoid even when oesophageal lesions compatible with mucous membrane pemphigoid were present at the time of biopsy.

Key words: mucous membrane pemphigoid; autoimmune bullous diseases; autoantibody; direct immunofluorescence microscopy; oesophagus; dysphagia.

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Corr: Enno Schmidt, Department of Dermatology, University of Lübeck, Lübeck, Germany, Ratzeburger Allee 160, DE-23562 Lübeck, Germany. E-mail: enno.schmidt@uksh.de

SIGNIFICANCE

The diagnostic gold standard for mucous membrane pemphigoid, a potentially fatal autoimmune blistering disease, is direct immunofluorescence microscopy of a perilesional biopsy. The aim of this study was to assess the diagnostic accuracy and clinical-immunopathological correlations of biopsies obtained from the oesophagus, as its epithelium represents a possible target of autoantibodies in mucous membrane pemphigoid. The results suggest that findings from direct immunofluorescence microscopy of oesophageal biopsies do not correlate with endoscopic findings and symptoms indicative for oesophageal involvement. Thus, these data favour oral or cutaneous perilesional biopsies for direct immunofluorescence microscopy even in the presence of oesophageal lesions and/or symptoms compatible with mucous membrane pemphigoid.

Mucous membrane pemphigoid (MMP) is a sub-epithelial autoimmune blistering disease defined by autoantibodies against structural proteins of dermal-epithelial junction (DEJ) and predominant mucosal involvement (1). Antigenic targets of IgG, and less frequently, IgA autoantibodies, include BP180 (type XVII collagen), laminin 332, BP230 (in conjunction with anti-BP180 reactivity), type VII collagen, and possibly $\alpha 6\beta 4$ integrin (2). Rarely, exclusive IgM reactivity has been described in MMP (3, 4). MMP represents a clinically and immunologically heterogeneous disorder potentially affecting several mucosal surfaces, e.g. oral cavity, conjunctivae, nostrils, larynx, pharynx, and anogenital area (5, 6). Oesophageal involvement is rare and may occur in 5–15% of patients (7). It may lead to severe strictures and stenosis, resulting in malnutrition, or even growth retardation in adolescent patients (8). Clinical symptoms of oesophageal involvement in MMP range from oral pain to dysphagia, odynophagia, and weight loss. Such MMP patients are generally recommended to undergo diagnostic oesophagogastroduodenoscopy (OGD) in order to establish or exclude oesophageal involvement (9).

The gold standard diagnostic procedure for MMP is direct immunofluorescence microscopy (DIF) of a

perilesional skin and/or mucosal biopsy in which linear deposits of IgG, IgA, IgM and/or complement C3 can be detected at the DEJ (5). A serious drawback, however, is the relatively low sensitivity of 50–70% in the initial biopsy for the diagnosis of MMP, which might entail multiple and repeated sampling (10, 11). To date, there is no consensus regarding if, and to what extent, oesophageal biopsies should be obtained as part of the routine diagnostic work-up in patients with clinical findings suggestive of MMP (2, 11). In previous studies, the diagnostic relevance of oesophageal DIF sampling in MMP has not yet been addressed systematically (8, 12, 13).

MATERIALS AND METHODS

Study cohort

This retrospective study included 11 patients with MMP who underwent OGD with concomitant oesophageal DIF sampling for diagnostic purposes, independent of pretreatment status. All biopsies were evaluated in the routine autoimmune diagnostic laboratory of the University of Lübeck, Germany, between 2006 and 2021. Diagnosis of MMP was made in accordance with current guidelines and based on: (i) clinical findings with predominant mucosal involvement, (ii) linear depositions of IgG and/or IgA and/or C3 at the DEJ by DIF, and/or (iii) detection of circulating autoantibodies against basement membrane zone (BMZ) by (a) indirect immunofluorescence microscopy (IIF) on monkey oesophagus and/or human salt-split skin, (b) enzyme-linked immunosorbent assay (ELISA) for IgG serum reactivity against BP180 NC16A, BP230, or type VII collagen (all Euroimmun, Lübeck, Germany), or (c) immunoblotting with concentrated conditioned medium of cultured HaCaT cells (for IgG and IgA against LAD-1, the soluble ectodomain of BP180), a C-terminal fragment of BP180 (BP180(ec)3, amino acids 1022 to 1266), or extracellular matrix of cultured HaCaT cells (for IgG4 reactivity against laminin 332) (11, 14–17).

Clinical and immunopathological data

Parameters included in this study, i.e. age, sex, clinical and endoscopic findings and immunopathological data, were retrieved from electronic medical records at the Department of Dermatology, University of Lübeck, Lübeck, Germany. Oesophageal involvement was established by OGD findings, as reported previously (9). Pathological oesophageal alterations suggestive of MMP were classified into active, i.e. erythema, blisters, erosions, ulcerations, and cicatricial lesions (i.e. strictures, fibrosis, stenosis, scarring with web formation), as described previously (8).

Statistical analysis

Fisher's exact test was used to compare 2 different groups. A p -value <0.05 was considered statistically significant. Statistical analyses were performed, and graphs were made using GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Demographic profile of the study cohort

Four female and 7 male patients were included. The mean age (\pm standard deviation; SD) at diagnosis was

77.3 (± 7.1) years. The median age of study participants was 80 years, ranging from 67 to 88 years (Table I).

Clinical features of the study population

All patients showed lesions within the oral cavity (100%), followed by ocular (in 8 patients), oesophageal (in 5 patients), pharyngeal (in 5 patients), laryngeal (in 4 patients) and nasal mucosae (in 3 patients) as well as the skin (in 3 patients). No involvement of the anorectal and genital mucous membranes was observed (Table I).

Oral pain was the most frequently reported symptom (in 10 patients). Dysphagia was present in 5 patients, occasionally accompanied by odynophagia, coughing, globus and dysgeusia (in 1 patient each) (Table SI). Unintentional weight loss occurred in 2 out of 3 patients who provided information on their weight status (Table I).

All patients underwent oesophageal biopsies during OGD performed due to their indicative symptoms. Five of these patients were under systemic immunosuppressive and/or immunomodulatory therapy at the time of OGD (Table SI). Overall, 5 patients showed macroscopic oesophageal findings compatible with MMP (Table SI). Within this group, 2 patients demonstrated exclusively active lesions (erythema, blisters, erosions and/or ulcerations), whereas solely cicatricial changes (strictures, fibrosis, stenosis, scarring with web formation) were detected in 1 patient. Concomitant presence of active and cicatricial lesions was identified in 2 patients (Fig. 1, Table I).

Table I. Demographic and clinical features of patients with mucous membrane pemphigoid (MMP)

Characteristics	
Age at diagnosis, years	
Mean (SD)	77.3 (7.1)
Median (range)	80 (67–88)
Sex, n (%)	
Female	4 (36.4)
Male	7 (63.6)
Involved anatomical sites, n (%)	
Oral	11 (100)
Ocular	8 (72.7)
Oesophageal	5 (45.5)
Pharyngeal	5 (45.5)
Laryngeal	4 (36.3)
Nasal	3 (27.3)
Cutaneous	3 (27.3)
Anogenital	0
Symptoms indicative of oesophageal involvement, n (%)	
Dysphagia	5/11 (45.5)
Oral pain	10/11 (90.9)
Weight loss	2/3 (66.7)
Others	3/11 (27.3)
OGD findings in line with oesophageal affection, n (%)	
Active lesions only	2/11 (18.2)
Cicatricial lesions only	1/11 (9.1)
Both active and cicatricial lesions	2/11 (18.2)

OGD: oesophagogastroduodenoscopy; SD: standard deviation.

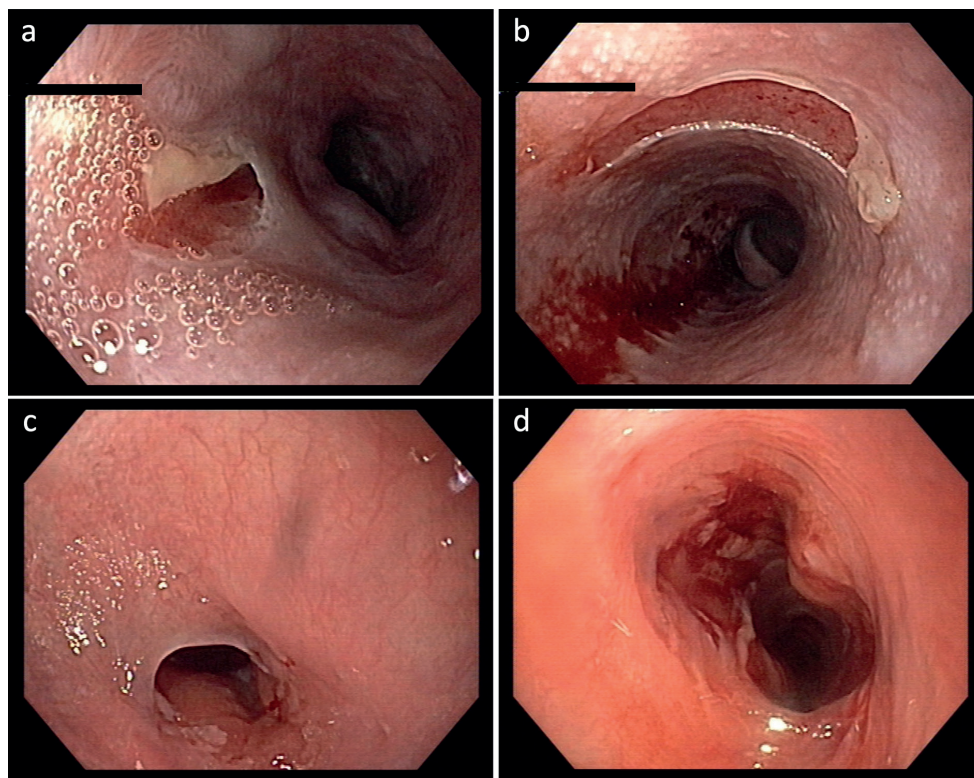


Fig. 1. Endoscopic images of the oesophagus showing (a, b) erosions of the mucous membrane in patient 7 and (c, d) demonstrating a stricture of the proximal oesophagus prior and following dilatation up to 14 mm in patient 6. Macroscopically, the findings were compatible with oesophageal involvement of mucous membrane pemphigoid, which was confirmed by direct immunofluorescence microscopy in both cases.

Table II. Immunopathological characteristics of mucous membrane pemphigoid (MMP) patients with and without oesophageal involvement

	Patients with oesophageal involvement (n = 5) n (%)	Patients without oesophageal involvement (n = 6) n (%)
Subsite-specific DIF positivity		
Oral	3/3 (100)	5/6 (83.3)
Oesophageal	1/5 (20)	1/6 (16.7)
Cutaneous	1/3 (33.3)	3/6 (50)
Ocular	1/1 (100)	0/1 (0)
Hypopharyngeal	1/1 (100)	n.d.
Gastric	n.d.	0/1 (0)
Duodenal	n.d.	0/1 (0)
Linear deposition of immunoreactants along the BMZ in DIF		
IgG	4/5 (80)	4/6 (66.7)
IgA	4/5 (80)	4/6 (66.7)
IgM	0/5 (0)	1/6 (16.7)
C3	2/5 (40)	2/6 (33.3)
Negative	1/5 (20)	0/6 (0)
IIF on human salt-split skin		
IgG	2/5 (40)	4/6 (66.7)
IgA	0/5 (0)	3/6 (50)
Epidermal side	1/5 (20)	5/6 (83.3)
Dermal side	1/5 (20)	0/6 (0)
Negative	3/5 (60)	1/6 (16.7)
Target antigen		
BP180 NC16A (IgG)	2/5 (40)	1/6 (16.7)
BP180(ec)3 (C-terminal, IgG)	1/5 (20)	0/6 (0)
BP180 4575 (C-terminal, IgG)	0/5 (0)	1/6 (16.7)
BP230 (IgG)	0/5 (0)	2/6 (33.3)
LAD-1 (IgA)	2/5 (40)	0/6 (0)
LAD-1 (IgG)	2/5 (40)	0/6 (0)
Laminin 332	1/5 (20)	0/6 (0)
Indeterminate	0/5 (0)	3/6 (50)

BMZ: basement membrane zone; DIF: direct immunofluorescence microscopy; IIF: indirect immunofluorescence microscopy; n.d.: not done.

Immunopathological aspects and evaluation of subsite-specific sampling

A cohort analysis of immunopathological characteristics was performed in patients with MMP and clinical symptoms suggestive of oesophageal affection. Of note, evaluation of DIF samples obtained from the oesophagus yielded no correlation with clinical oesophageal involvement ($p=1.000$; OR 1.250; 95% confidence interval (95% CI) 0.05811–26.89) (Table II). No significant difference with regard to oesophageal DIF positivity was highlighted between patients with vs without systemic therapy at the time of OGD ($p=1.000$; OR 0.5769; 95% CI 0.03728–5.494). Subsequently, pooled analysis of all 67 biopsies obtained from 11 patients with MMP was conducted, including, inter alia, 31 oesophageal, 16 oral, and 11 cutaneous biopsies. Overall, perilesional oral biopsies showed significantly more positive DIF results than oesophageal biopsies in all patients with MMP ($p<0.0001$; OR 0.03571; 95% CI 0.008907–0.1847). A parallel, but less pronounced, tendency was observed for cutaneous biopsies in comparison with oesophageal samples ($p=0.0195$; OR 0.01286; 95% CI 0.02393–0.6909) (Table III). In this regard, DIF positivity was identified in 5 skin biopsies obtained from 4 patients, of which 2 had cutaneous involvement of MMP (Table SI). On the other hand, no significant difference was found in terms of DIF positivity between oral and cutaneous

Table III. Site-specific pooled analysis of all samples obtained from patients with mucous membrane pemphigoid (MMP)

	Linear deposits of immunoreactants in DIF <i>n</i> (%)	Negative DIF <i>n</i> (%)
Biopsy site		
Oral (<i>n</i> =16)	12 (75)	4 (25)
Cutaneous (<i>n</i> =11)	5 (45.5)	6 (54.5)
Ocular (<i>n</i> =3)	2 (66.7)	1 (33.3)
Oesophageal (<i>n</i> =31)	3 (9.6)	28 (90.3)
Upper third (<i>n</i> =5)	3 (60)	2 (40)
Middle third (<i>n</i> =6)	0	6 (100)
Lower third (<i>n</i> =1)	0	1 (100)
Oesophagus, not specified (<i>n</i> =19)	0	19 (100)
Hypopharyngeal (<i>n</i> =2)	1 (50)	1 (50)
Duodenal (<i>n</i> =2)	0	2 (100)
Gastric (<i>n</i> =2)	0	2 (100)

DIF: direct immunofluorescence microscopy.

biopsies in this study population ($p=0.2238$; OR 0.2778; 95% CI 0.05387–1.432) (Table III).

Subsite-specific distribution of *in vivo* bound immunoreactants by DIF revealed a significantly higher prevalence of IgG deposits in oral as well as cutaneous biopsies compared with oesophageal samples ($p<0.0001$; OR 50.00; 95% CI 5.348–467.4, and $p=0.0126$; OR 17.4; 95% CI 1.649–178.2, respectively). Oral samples also showed significantly more IgA deposits than biopsies obtained from the oesophagus ($p=0.0036$; OR 9.333; 95% CI 1.996–43.64). On the contrary, no significant difference was seen between cutaneous and oesophageal samples with regards to IgA deposits ($p=0.0635$; OR 5.333; 95% CI 0.9634–29.52) (Fig. 2).

Serological studies revealed IgG autoantibodies against the 16th non-collagenous domain (NC16A) of BP180 in 2 patients with oesophageal involvement. Similarly, the soluble ectodomain of BP180 (LAD-1) was recognized by IgG and IgA autoantibodies in 2 patients. IgG autoantibodies recognizing a C-terminal stretch of BP180 (BP180(ec)3), and laminin 332 were found in 1 patient each. Autoantibodies against BP230 were not detected in this cohort, while BP230 was the most frequently targeted epitope in patients without oesophageal affection (in 2 patients), followed by BP180 NC16A (in 1 patient) and a C-terminal domain of BP180 (BP180 4575) (in 1 patient) (Table II).

DISCUSSION

Detection of tissue-bound autoantibodies against BMZ by DIF is regarded as the major diagnostic criterion of MMP and other pemphigoid diseases (1, 5). Reports regarding the sensitivity of DIF in MMP have been inconclusive, demonstrating a wide range from 41% to 100% for mucosal biopsies (10, 18–21), and 44% to 100% for skin samples (10, 18, 20, 21). Notably, in previous studies, circulating autoantibodies have been detected in only 2.6% to 8% of patients with MMP using monkey oesophagus substrate (22–24), and in 36% to 84% with 1 M sodium chloride-split normal human skin (15, 23, 25–29). Owing to the comparably low sensitivity of DIF and IIF (11), the definitive diagnosis of MMP has posed a core challenge. As demonstrated by Shimanovich et al. (10), multiple and repeated sampling may be required to increase the sensitivity of DIF in MMP.

In this retrospective study, only 3 out of 31 biopsies obtained from oesophagus (9.6%) showed linear deposits of immunoreactants (Table III). This corroborates with previous findings by Zehou et al. (8), in which all oesophageal DIF samples from 5 patients with MMP and established oesophageal involvement were negative or not interpretable. In contrast, a study reported 5 patients with MMP who had endoscopic findings compatible with oesophageal involvement, and positive DIF staining of perilesional oesophageal biopsies (12). However, this can be attributed to its selection criteria, as only patients with positive oesophageal DIF results were included. Of note, that study found a heterogeneous anti-BMZ response for IgA and, to a lesser extent, for IgG in MMP with oesophageal involvement. This concurs well with the current data regarding an IgA-predominant mixed autoantibody response in positive oesophageal DIF samples (Fig. 2). In this context, a dual circulating autoreactivity of IgA and IgG has been found to be associated with a more severe disease profile in MMP than an exclusively IgG-mediated disease (29). Remarkably, the current study could identify antigenic targets in all 5 patients with affection of the oesophagus, whilst autoantigens

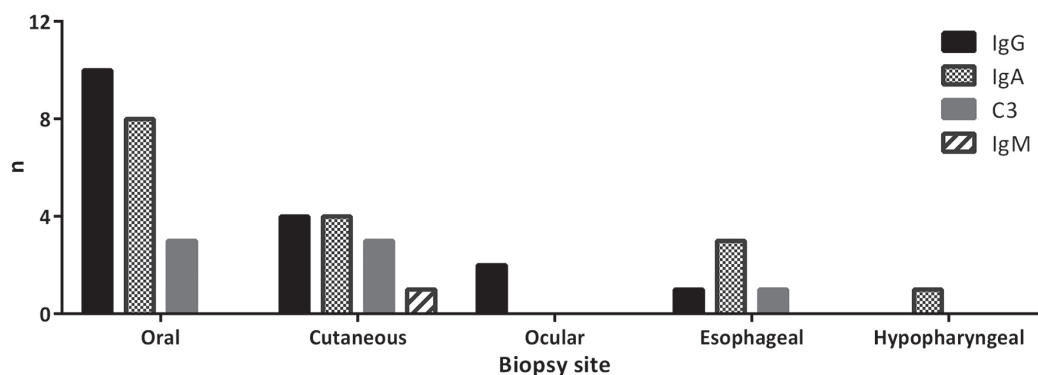


Fig. 2. Site-specific distribution of tissue-bound autoantibodies in direct immunofluorescence microscopy, based on pooled analysis of all samples from 11 patients with mucous membrane pemphigoid (MMP). Total number of biopsies from oral cavity ($n=16$), skin ($n=11$), eyes ($n=3$), oesophagus ($n=31$), hypopharynx ($n=2$). Ig: immunoglobulin.

remained elusive in half of the patients without oesophageal alterations (Table II). This could possibly be attributed to higher levels of autoantibodies in the former cohort, leading to severe disease phenotype with oesophageal involvement, whereas low serum antibody levels in the latter group might remain undetectable. Anti-LAD-1 IgG and IgA autoantibodies as well as anti-laminin 332 IgG autoantibodies were found in 2, 2, and 1 patient(s) with oesophageal involvement, respectively, but in none of the patients in the other cohort. Conversely, BP230 was recognized by autoantibodies in one-third of the patients lacking oesophageal lesions, while being absent in patients exhibiting those (Table II). Due to the small sample size, no definite association can be determined between a specific target antigen and oesophageal involvement in MMP.

The optimal biopsy location for DIF assay in MMP has been a point of controversy for many years. Recent evidence suggests that punch biopsy from uninvolved buccal mucosa is as sensitive as perilesional cutaneous biopsy, and superior to gingival biopsies (30). The current study adds to a growing body of research on this issue, demonstrating that oral and cutaneous biopsies led to a significantly higher rate of positive DIF results in patients with MMP compared with oesophageal biopsies. Notably, in the current study, tissue-bound autoantibodies were detected only in the proximal third of the oesophagus (Table III). This is in line with a previous study, which demonstrated that active oesophageal lesions were found predominantly in the upper third segment in patients with MMP (8). This predilection may be due to the transition from stratified squamous epithelium in the upper oesophagus, resembling that in the oral mucosa, to single-layered columnar epithelia in the lower parts. However, further investigations are needed to estimate the expression levels of structural DEJ proteins in different parts of the oesophagus.

Oesophageal involvement is considered one of the most life-threatening, "high-risk" manifestations of MMP, affecting approximately 5–15% of patients with MMP (1, 7, 8, 31). Several experts have recommended endoscopic examination in symptomatic patients to allow early diagnosis and prevent irreversible complications (8, 9). In this study, no correlation was found between oesophageal involvement in MMP and DIF results of oesophageal samples. Future studies are required to validate these results by a larger sample size. Since oesophageal biopsies are not performed on a regular basis in patients with MMP, a multicentric approach could provide an extended patient cohort.

In conclusion, the current data suggest that oesophageal samples are inferior to oral and cutaneous biopsies in the diagnosis of MMP. Therefore, oesophageal biopsies should be performed only after careful benefit-risk assessment.

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IRB approval status: The study adheres to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Lübeck (22-074).

The authors have no conflicts of interest to declare.

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