Insights in clinical and diagnostic findings and treatment responses in patients with mucous membrane pemphigoid, a retrospective cohort study.

Hanan Rashid, MD, Joost M. Meijer, MD PhD, Maria C. Bolling, MD PhD, Gilles F.H. Diercks, MD PhD, Hendri H. Pas, PhD, Barbara Horváth, MD PhD

PII: S0190-9622(21)02958-3
DOI: https://doi.org/10.1016/j.jaad.2021.11.061
Reference: YMJD 16519

To appear in: Journal of the American Academy of Dermatology

Received Date: 10 May 2021
Revised Date: 3 November 2021
Accepted Date: 22 November 2021


This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier on behalf of the American Academy of Dermatology, Inc.
Insights in clinical and diagnostic findings and treatment responses in patients with mucous membrane pemphigoid, a retrospective cohort study.

Hanan Rashid MD, Joost M. Meijer MD PhD, Maria C. Bolling MD PhD, Gilles F.H. Diercks MD PhD, Hendri H. Pas PhD, Barbara Horváth MD PhD

1 University of Groningen, University Medical Center Groningen, Department of Dermatology, Center of Blistering Diseases, European Reference Network-Skin Member, Groningen, the Netherlands

2 University of Groningen, University Medical Center Groningen, Department of Pathology, Groningen, the Netherlands

Corresponding author:
Hanan Rashid
Department of Dermatology, University Medical Center Groningen
Hanzeplein 1
9700 RB Groningen
The Netherlands
tel: +31-50-3610701 fax: +31-503612624
e-mail: h.rashid@umcg.nl

Funding sources: None

Conflicts of Interest:
B. Horváth reports fees from Janssen-Cilag (Advisory Boards, Educational grants, Consultations, Investigator Initiative Studies), AbbVie (Advisory Boards, Educational grants, Consultations, Investigator Initiative Studies), Novartis Pharma (Advisory Boards, Consultations, Investigator Initiative Studies), UCB Pharma (Advisory Boards, Consultations), Leo Pharma (Consultations), Solenne B.V. (Investigator Initiative Studies), Celgene (Consultations, Investigator Initiative Studies), Akari therapeutics (Consultations, Investigator Initiative Studies), Philips (Consultation), Roche (Consultation), Regeneron (Consultation) and Sanofi (Consultation). The remaining authors have no conflict of interest to declare.

IRB approval status: Reviewed and approved by IRB; approval 2018/530

Manuscript word count: 2500 words [excluding capsule summary, abstract, references, figures, tables]

Abstract word count: 200

Capsule summary word count: 50

References: 41

Figures: 4

Supplemental figures: 0

Tables: 1

Supplemental tables: 4

Mendeley Link: https://data.mendeley.com/datasets/dm356kv35j/1
Keywords: immunology, autoimmune bullous diseases, autoimmune blistering disease, mucous membrane pemphigoid, case series, clinical characteristics, laminin-332, malignancy
Abstract

Background: The variable clinical severity of MMP often leads to a diagnostic and therapeutic delay.

Objective: To describe the characteristics in a large cohort of patients with MMP.

Methods: A retrospective review study of clinical and diagnostic characteristics and treatment response in 145 patients with MMP.

Results: Monosite involvement was seen in 41.4% and multisite involvement in 58.6% patients. The oral mucosa was affected in 86.9%, followed by the ocular mucosa (30.3%), skin (26.2%), genital mucosa (25.5%), nasal mucosa (23.4%) and pharyngeal and/or laryngeal mucosa (17.2%). Ocular disease developed during disease course in 41.7% of patients with initially other mucosal site involvement. The malignancy rate was significantly higher in patients with autoantibodies against laminin-332 compared to MMP patients without laminin-332 autoantibodies (35.3% vs. 10.9%, p=0.007). Systemic immunosuppressive or immunomodulatory therapy were administered in 77.1% of the patients, mainly in patients with multisite involvement (p<0.001), ocular involvement (p<0.001) and pharyngeal and laryngeal involvement (p=0.002). The remaining patients (22.9%) received topical therapy. Adverse events were frequently reported.

Limitations: retrospective design.

Conclusion: MMP presents with a heterogeneous clinical presentation and new symptoms may develop during the disease course. Cancer screening should be considered in MMP and in particular with autoantibodies against laminin-332.
Capsule summary

- Mucous membrane pemphigoid (MMP) is a group of rare autoimmune bullous diseases with highly variable clinical heterogeneity and a potential diagnostic delay.
- Patients with MMP may develop lesions of other mucosal sites during follow-up. Clinicians should be aware of a potential occurrence of malignancies in MMP with autoantibodies against laminin-332.
INTRODUCTION

Mucous membrane pemphigoid (MMP) comprises a group of heterogeneous autoimmune blistering diseases with predominant mucosal involvement characterized by autoantibodies directed against structural proteins in the epidermal basement membrane zone (EBMZ) including BP180, BP230, laminin-332, type VII collagen and α6 and β4 integrin subunits. Involvement of one or more mucosal sites may occur, making MMP a clinically heterogeneous disease. Various terms were previously used to define MMP including benign mucous membrane pemphigoid and (ocular) cicatricial pemphigoid.

According to The First International Consensus on MMP, and the recent published European guideline on MMP, the term MMP is the appropriate terminology for this entity and older terminology should be avoided. The clinical severity of MMP is highly variable ranging from mild oral inflammation or conjunctivitis, to severe complications such as progressive conjunctival cicatrization that may culminate in blindness, or life-threatening laryngeal obstruction without treatment.

The diagnosis of MMP is based on a mucosal and/or skin biopsy for direct immunofluorescence microscopy (DIF) detecting linear deposition of autoantibodies along the EBMZ. In addition, indirect immunofluorescence microscopy (IIF) on human salt-split skin (SSS) and several immunoserologic tests can detect circulating autoantibodies in serum. Systemic immunosuppressive and immunomodulatory drugs are part of the management of MMP and can be administered as monotherapy or combined, depending on the severity.

Large retrospective case studies on MMP are scarce and have been mainly performed in patients with oral or ocular involvement. The aim of this study was to describe the clinical and diagnostic findings and treatment responses of patients diagnosed with different subtypes of MMP to support early recognition and improve patient care.
MATERIALS AND METHODS

Study design

This single-centre retrospective study included patients diagnosed with MMP between 2002 and 2019 at the Center for Blistering Diseases in Groningen, the Netherlands. Eligible participants were included according to previously described criteria. Laboratory techniques and interpretation of DIF, IIF on SSS, immunoblot and ELISA were performed as previously described. The keratinocyte footprint assay (KFA) was retrospectively performed in all patients’ sera.

Clinical characteristics were assessed by reviewing patient medical records. Treatment response and adverse effects of the following therapies were collected: dapsone, cyclophosphamide, azathioprine, mycophenolic acid and rituximab. Only patients who received these as monotherapy or in combination with short term prednisone were assessed. The early and late clinical outcomes disease control (DC) and remission (partial or complete) defined by international consensus, were used. Data were collected anonymously in electronic case report forms using OpenClinica software (OpenClinica, Waltham, MA). Individuals were excluded in case of a diagnosis of cutaneous pemphigoid and if accurate clinical details were missing. The study was approved by the University Medical Center Groningen Medical Ethical Committee. Written consent for the images was provided.

Statistical analysis

All continuous outcomes were described as median with interquartile range (IQR). Correlations between bivariate outcome measures were analyzed using the Chi squared test or Fisher’s exact test. Comparing means or medians of unpaired continuous data were analyzed with Mann–Whitney U test. Binary logistic regression was performed to analyze predictors of malignancy. Statistical significance was defined by a p-value <0.05. Statistical analyses were performed in SPSS Statistics, version 23 (IBM).
RESULTS

Patient characteristics

Overall, 145 patients diagnosed with MMP were included. The median age at diagnosis in years was 64.0 (IQR 18.0), with a female predominance (n=84, 57.9%). In 60 patients (41.4%), involvement of one mucosal site was seen, opposing 85 patients (58.6%) with multisite involvement. The median diagnostic delay in months was 12.0 (IQR 21.8). No significant difference was seen in the diagnostic delay between monosite and multisite involvement (median 13.5 IQR 24.5 vs. 9.0 IQR 19.0 respectively, p=0.088). The median follow-up time in months was 26.0 (IQR 40.0, n=129).

Clinical findings

Table I presents an overview of the mucosal lesions and symptoms per mucosal site. The majority of patients with MMP presented with involvement of the oral mucosa (n=126, 86.9%), followed by the ocular mucosa (n=44, 30.3%), genital mucosa (n=37, 25.5%), nasal mucosa (n=34, 23.4%) and pharyngeal and/or laryngeal mucosa (n=25, 17.2%). The diagnosis of pharyngeal and/or laryngeal MMP was made by an otorhinolaryngologist through nasendoscopy and laryngoscopy. Skin involvement was reported in 38 MMP patients (26.2%) of which 11 MMP patients presented with skin lesions preceding mucosal lesions, whereas in nine MMP patients skin lesions developed after mucosal symptoms. Moreover, 15 out of 36 (41.7%) of the patients with ocular multisite involvement developed the ocular symptoms after a median months of 48.0 (IQR 71.0) of first reported symptoms. Patients were referred to the ophthalmology in the presence of ocular symptoms to confirm diagnosis. The clinical characteristics of patients with autoantibodies against laminin-332 (n=17, 11.7%) consisted of involvement of the oral mucosa (n=17), nasal cavity (n=10), conjunctivae (n=9), pharynx (n=5), larynx (n=5) and genital mucosa (n=2). Multisite involvement was more frequently seen in this subgroup (n=15, 88.2% vs. n=70, 54.7% p=0.018, Mendeley supplemental table I) as well as a male predominance (n=13, 76.5% vs. n=58, 37.5%
A malignancy was reported in 20/145 MMP patients (13.8%), mainly solid tumors, of which 9 patients (6.2%) developed the malignancy during follow-up (median 26.5 months, IQR 74.3). These malignancies included lung carcinoma (n=3), prostate cancer (n=1), penile cancer (n=1), breast cancer (n=1), endometrial cancer (n=1), vulvar carcinoma (n=1) and Non-Hodgkin lymphoma (n=1). Notably, 6 out of 17 patients with autoantibodies against laminin-332 (35.3%) had a malignancy, compared to 14 out of 128 patients (10.9%) without these autoantibodies (OR 6.08; 95% CI 1.65-22.44, p=0.007) adjusted for age, gender and use of systemic treatment (Mendeley supplemental table I).

Direct immunofluorescence microscopy and histopathology

A biopsy for DIF from perilesional or healthy mucosa and/or skin was performed in all 145 MMP patients and showed linear depositions of autoantibodies in 137 MMP patients (94.5%). Linear IgG deposition was seen in the majority of MMP patients (n=118, 81.4%), in contrast to IgA (n=82, 57.0%) and C3c (n=80, 55.2%). Interestingly, linear deposition of IgA, alone or combined with IgG/C3c, was more often seen in patients with multisite involvement compared with monosite involvement (n=55, 65.5% vs. n=27, 45.8%; p=0.002) and in patients with ocular involvement compared with those without ocular involvement (n=32, 72.7% vs. n=58, 54.7%; p=0.013). Linear IgA deposition was also seen more often in MMP patients who received systemic treatment compared to those with local treatment (n=66, 61.7% vs. n=12, 38.7%; p=0.043). Histopathology showed a subepithelial split in 56 biopsies (48.7%). Furthermore, eosinophils were observed in 40 biopsies (34.8%) and ulceration or erosions in 33 biopsies (29.0%).

Indirect immunofluorescence microscopy and serology
IIF on SSS was performed in all 145 MMP patients and showed a positive result in 65 patients (44.8%), of which 35 patients showed epidermal binding of IgG (53.8%), eight with IgA (12.3%) and nine combined IgG and IgA (13.8%). Dermal binding of IgG and/or IgA was observed in 13 patients (9.0%). KFA was performed in all 145 MMP patients, of which 17 (11.7%) showed autoantibodies against laminin-332. IIF on SSS was positive in 12/17 of these patients: three with epidermal and nine with dermal binding. Mendeley supplemental table II summarizes the results of immunoblot and ELISA.

**Treatment response**

During follow-up, the majority (n=108, 77.1%) received systemic immunosuppressive or immunomodulatory therapy, with a median number of 2 (IQR 3.5) systemic therapies while the remaining patients (n=37, 22.9%) received topical therapy, mainly high potency corticosteroids. Notably, patients with multisite involvement more often received systemic therapy compared with those with monosite involvement (n=73, 89.0% vs. n=35, 60.3%; p<0.001). In addition, patients with ocular involvement and patients with pharyngeal involvement alone or combined with laryngeal involvement more often received systemic therapy compared with those without these involvements (n=40, 97.6% vs. n=68, 68.7%; p<0.001 and n=24, 100% vs. n=84, 72.4%; p=0.002, respectively). Mendeley supplemental table III summarizes the characteristics of patients with MMP and their response to immunosuppressive and immunomodulatory treatment. The majority had multisite involvement. A total of 72 patients received dapsone, of which 31 patients achieved DC (43.1%) and 25 patients remission (34.7%). Cyclophosphamide was administered in 44 patients, of which 21 patients achieved DC (47.7%) and 15 patients (34.1%) remission. Only 6 (22.2%) and 5 (18.5%) patients achieved DC and remission with azathioprine (n=27) respectively, and 4 (33.3%) and 2 (16.7%) patients with mycophenolic acid (n=12). Finally, 28 patients received rituximab, of which 18 patients (64.3%) achieved DC and 15 patients
(53.6%) remission. The reason of cessation of therapy was mainly because of side-effects or ineffectiveness. Almost all patients reported adverse events during therapy (Mendely supplemental Table IV).
DISCUSSION

MMP is a chronic group of diseases with a substantial diagnostic delay, mainly due to the highly variable clinical phenotype. This large cohort study of well-defined MMP patients sought to improve our understanding of the clinical characteristics of MMP. MMP may affect different sites during the disease course. Our results showed that nearly 42% of patients with ocular multisite involvement developed ocular symptoms later in the disease course. Previously, Higgins et al. reported the development of ocular disease in 37% of patients with oral MMP. Therefore, follow-up care including regular physical examination and consultation of an ophthalmologist at first visit and during follow-up in case of clinical symptoms, is essential in order to prevent suboptimal treatment and complications. Factors contributing to scar formation in MMP are poorly understood. Inflammatory responses near the lamina densa and papillary dermis may contribute to scar formation. In this study, scarring was mainly seen in patients with ocular, pharyngeal and laryngeal involvement and was less common in patients with oral involvement, although during the healing phase fibrosis may be observable in the oral mucosa.

Several reports have shown that a mucosal or skin biopsy for DIF yields the highest sensitivity for diagnosis of MMP, in contrast to IIF on SSS. Histopathology showed in less than half of the patients a subepithelial split and is therefore not a useful tool for diagnosis of MMP. In this study, a positive biopsy for DIF was seen in 94.5% and a positive SSS in 44.8% of the patients. Interestingly, deposition of linear IgA in DIF and combined IgA and IgG in IIF on SSS were more frequently seen in patients with multisite involvement compared with those with monosite involvement. In addition, patients with ocular involvement showed more frequently linear deposition of IgA than those without ocular involvement. We also identified that MMP patients with IgA depositions more often required systemic therapy. A previous study has shown that combined IgA and IgG reactivity was associated with a more severe disease compared with the presence of IgG alone. It is postulated that IgA, possibly
together with IgG may activate a complement-mediated inflammatory response, resulting in progression of mucosal lesions in MMP.26

The pathogenic role of autoantibodies in MMP has been shown both in vitro and in vivo. In more than half of the patients we found a positive immunoblot of which the majority with IgG or IgA against BP180. Previous studies have confirmed that BP180 is the most frequent target antigen in MMP, detected in the majority of MMP sera.27–29 Laminin-332 is targeted by a subset of MMP patients, often with multisite involvement including pharyngo-laryngeal and oro-pharyngolaryngeal involvement.30,31 Our results showed that 17/145 patients had autoantibodies against laminin-332 detected by KFA, a specific test to assess the presence of these autoantibodies.11 Of these, only 9 showed dermal binding by IIF SSS and 5 had a negative IIF SSS. Noteworthy, the remaining 3 patients showed only an epidermal binding, indicating combined presence of autoantibodies such as laminin-332 and BP180. With KFA we were able to detect more patients with autoantibodies against laminin-332 compared to IIF SSS, suggesting that it is a sensitive technique. The potential occurrence of a malignancy in MMP, and in particular patients with autoantibodies against laminin-332, is a matter of controversy. Previous studies showed conflicting results on the association of malignant neoplasms in MMP with autoantibodies against laminin-332.32–35 The rarity of this disease and possible confounders such as age, use of immunosuppressive therapy and the applied detection technique for laminin-332 autoantibodies may influence these results. In our series, the malignancy rate was significantly higher in patients with autoantibodies against laminin-332 (35.3%) compared to MMP patients without antibodies against laminin-332 (10.9%). Future prospective multicenter studies are needed to assess the risk and etiology of malignancies in MMP. However, clinicians should be aware of a potential occurrence of malignancies in MMP and in particular those with autoantibodies against laminin-332. Therefore, oncological screening is recommended, in particular for solid tumors, at initial diagnosis and on indication during follow-up.4
Systemic immunosuppressive and immunomodulatory therapy is often required to cease the inflammation and stop the progression of scarring. In this study, patients with multisite involvement and patients with ocular involvement more often required systemic therapy, indicating a more severe disease course. Moreover, involvement of the pharynx or larynx was also associated with the administration of systemic therapy, as these locations are difficult to reach with topical treatment. Previous reports have reported satisfactory results of dapsone, cyclophosphamide, azathioprine and mycophenolic acid in MMP, depending on the severity. We found rather poor results with remission percentages between 16% and 35% with and without short term prednisone. However, a direct comparison of endpoints is difficult, as several studies used different outcome measures. Patients and clinicians should be aware of the side effect profile of these immunosuppressive drugs. Rituximab, a monoclonal anti-CD20 antibody, is increasingly used in patients with refractory MMP. Our data showed a remission rate of 54% in patients treated with different dosages of rituximab. Previously, Lamberts et al. evaluated the effectiveness of rituximab in MMP in more detail in our center, showing partial remission in 65% and complete remission in 29% of the patients, but with a high relapse rate (75%). Other biologics, such as intravenous immunoglobulin and TNF-alfa inhibitors are used in recalcitrant cases and are considered as third- and fourth-line therapy, respectively. Further prospective studies are needed to position and compare these drugs in the treatment of MMP.

Strengths of this study include the considerable sample size and the comprehensive clinical data. Limitations of this study were the retrospective design and the lack of disease severity measurements such as the Mucous Membrane Pemphigoid Disease Area Index and patient reported outcome measures during follow-up. Long-term follow-up is critical to provide longitudinal data on treatment outcome and alterations in the clinical course of this chronic disease. However, our data provided relevant data on early and late endpoints of the administered systemic therapy during a median time of 26 months. In summary, symptoms of other mucosal sites in MMP, such as ocular disease may develop during the
disease course. Therefore, MMP patients should be regularly monitored in a multidisciplinary team. Due to the possible increased risk for malignancy in MMP and in particular with autoantibodies against laminin-332, cancer screening should be considered in these patients. Systemic immunosuppressive or immunomodulatory therapy were mainly administered in patients with multisite involvement, ocular, pharyngeal and laryngeal involvement. The rather moderate treatment response and frequent adverse events emphasize the need of early recognition, diagnosis and prevention of the progressive disease course of MMP.
List of abbreviations:

- CI: Confidence interval
- DIF: Direct immunofluorescence microscopy
- EBMZ: Epidermal basement membrane zone
- ELISA: Enzyme-linked immunosorbent assay
- IIF: Indirect immunofluorescence microscopy
- KFA: Keratinocyte footprint assay
- MMP: Mucous membrane pemphigoid
- OR: Odds ratio
- SSS: Salt-split skin substrate
- IQR: Interquartile range


17. Williams GP, Radford C, Nightingale P, Dart JKG, Rauz S. Evaluation of early and late presentation of
patients with ocular mucous membrane pemphigoid to two major tertiary referral hospitals in the

18. Benzaquen M, Suter VGA, Gschwend M, Feldmeyer L, Borradori L. Mucous membrane pemphigoid of
the oral lichen type: a retrospective analysis of 16 cases. *J Eur Acad Dermatology Venereol* 2019;33:e205-
207.


20. Chan LS. Immune-Mediated Subepithelial Blistering Diseases of Mucous Membranes. *Arch Dermatol*
1993;129:448.


epitopes on both intra- and extracellular domains of bullous pemphigoid antigen 180. *Br J Dermatol*

antibody response with IgG and IgA signifies a more severe and persistent disease. *Br J Dermatol*

extracellular domains are major autoantigens in mucous membrane pemphigoid: The pathogenic
relevance to HLA class II alleles and disease severity. *Br J Dermatol* 2006;154:90–8.

27. Cozzani E, Di Zenzo G, Calabresi V, et al. Autoantibody Profile of a Cohort of 78 Italian Patients with
Mucous Membrane Pemphigoid: Correlation Between Reactivity Profile and Clinical Involvement. *Acta
Derm Venereol* 2016;96:768–73.

Antibodies in Patients With Mucous Membrane Pemphigoid Predominantly Affecting the Oral Cavity. *J


30. Amber KT, Bloom R, Hertl M. A systematic review with pooled analysis of clinical presentation and
immunodiagnostic testing in mucous membrane pemphigoid: Association of anti-laminin-332 IgG with
oropharyngeal involvement and the usefulness of ELISA. *J Eur Acad Dermatology Venereol* 2016;30:72–
7.


Figure legend

Figure 1. Clinical characteristics of patients with mucous membrane pemphigoid (A) Gingivitis (B) symblepharon (C) laryngeal cicatrization and erosions (D) vulvar erosions, fusion of the labia and architecture loss.
Table legend

Table I. Clinical findings and symptoms of patients with mucous membrane pemphigoid

<table>
<thead>
<tr>
<th>Location of lesions</th>
<th>Oral mucosa n=126</th>
<th>N (%)*</th>
<th>Ocular mucosa n=44</th>
<th>N (%)*</th>
<th>Nasal mucosa n=34</th>
<th>N (%)*</th>
<th>Pharyngeal and/or laryngeal mucosa n=25</th>
<th>N (%)*</th>
<th>Genital mucosa n=37</th>
<th>N (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosite involvement</td>
<td>44 (34.9)</td>
<td></td>
<td>Monosite involvement</td>
<td>8 (18.2)</td>
<td>Monosite involvement</td>
<td>0 (0)</td>
<td>Monosite involvement</td>
<td>0 (0)</td>
<td>Monosite involvement</td>
<td>8 (21.6)</td>
</tr>
<tr>
<td>Multisite involvement</td>
<td>82 (65.1)</td>
<td></td>
<td>Multisite involvement</td>
<td>36 (81.2)</td>
<td>Multisite involvement</td>
<td>34 (100)</td>
<td>Multisite involvement</td>
<td>25 (100)</td>
<td>Multisite involvement</td>
<td>29 (78.4)</td>
</tr>
</tbody>
</table>

**Location of lesions**

**Clinical findings**

- **Erosions**: 93 (75.6)
- **Blisters**: 60 (48.8)
- **Erythema**: 54 (43.9)
- **Gingivitis**: 34 (27.6)
- **White lines**: 22 (17.9)
- **Ulceration**: 16 (13.0)
- **Fibrosis**: 3 (2.4)

**Symptoms**

- **Pain**: 56 (59.6)
- **Difficulty eating**: 36 (38.3)
- **Pain swallowing**: 28 (29.8)
- **Bleeding**: 27 (28.7)
- **Redness**: 17 (18.1)
- **Peeling**: 12 (12.8)
- **Discomfort**: 8 (8.5)
- **Burning**: 5 (5.3)

* Percentages were calculated after exclusion of patients for which data were unknown.
Capsule summary

- Mucous membrane pemphigoid (MMP) is a group of rare autoimmune bullous diseases with highly variable clinical heterogeneity and a potential diagnostic delay.
- Patients with MMP may develop lesions of other mucosal sites during follow-up. Clinicians should be aware of a potential occurrence of malignancies in MMP with autoantibodies against laminin-332.