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GUIDELINES

European Guidelines (S3) on diagnosis and management of mucous membrane pemphigoid, initiated by the European Academy of Dermatology and Venereology – Part II

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Abstract

This guideline has been initiated by the task force *Autoimmune Blistering Diseases* of the European Academy of Dermatology and Venereology, including physicians from all relevant disciplines and patient organizations. It is a S3 consensus-based guideline that systematically reviewed the literature on mucous membrane pemphigoid (MMP) in the MEDLINE and EMBASE databases until June 2019, with no limitations on language. While the first part of this guideline addressed methodology, as well as epidemiology, terminology, aetiology, clinical presentation and outcome measures in MMP, the second part presents the diagnostics and management of MMP. MMP should be suspected in cases with predominant mucosal lesions. Direct immunofluorescence microscopy to detect tissue-bound IgG, IgA and/or complement C3, combined with serological testing for circulating autoantibodies are recommended. In most patients, serum

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autoantibodies are present only in low levels and in variable proportions, depending on the clinical sites involved. Circulating autoantibodies are determined by indirect IF assays using tissue substrates, or ELISA using different recombinant forms of the target antigens or immunoblotting using different substrates. The major target antigen in MMP is type XVII collagen (BP180), although in 10–25% of patients laminin 332 is recognized. In 25–30% of MMP patients with anti-laminin 332 reactivity, malignancies have been associated. As first-line treatment of mild/moderate MMP, dapsone, methotrexate or tetracyclines and/or topical corticosteroids are recommended. For severe MMP, dapsone and oral or intravenous cyclophosphamide and/or oral corticosteroids are recommended as first-line regimens. Additional recommendations are given, tailored to treatment of single-site MMP such as oral, ocular, laryngeal, oesophageal and genital MMP, as well as the diagnosis of ocular MMP. Treatment recommendations are limited by the complete lack of high-quality randomized controlled trials.

Conflict of interest

Funding source

Diagnostics

Diagnosis of mucous membrane pemphigoid (MMP) is based on clinical findings (see part I) together with detection of anti-basement membrane zone (BMZ) autoantibodies. These autoantibodies are tissue-bound, detected by direct immunofluorescence (DIF) microscopy and/or direct immunoelectron microscopy, or circulating when detected either by indirect IF (IIF), ELISA or immunoblotting. Histopathology may be helpful in some cases when MMP, or another autoimmune blistering disease (AIBD), cannot be detected using these methods. About 50% of cases of ocular MMP cannot be confirmed by BMZ autoantibody detection tests. To exclude other cicatrizing conjunctival disorders with a similar disease course, this subset of ocular MMP cases requires an additional panel of investigations before a diagnosis of ocular MMP can be confirmed (see section on Diagnosis of ocular MMP).

Direct immunofluorescence microscopy

Direct immunofluorescence visualizes *in vivo* bound immunoreactants in skin or mucosa and shows linear deposition of IgG and/or IgA and complement C3 along the BMZ in MMP. DIF of a perilesional biopsy is considered the reference standard for diagnosis of MMP.[1] Sensitivities have been reported in a wide range, between 41 and 100%, depending on biopsy site. Mainly retrospective studies have been performed to assess the diagnostic accuracy of DIF, reporting high sensitivities when DIF is used as the reference standard for diagnosis, and lower sensitivities when clinical criteria have been used. Highest sensitivity has been found in MMP whereby both mucosa and skin were affected.[2] DIF biopsies of mucosa have been reported to have sensitivities between 41 and 100%,[2–7] and of skin between 44 and 100%.[2,3,6–11]

Conclusions

Level of evidence 2	DIF is the major diagnostic test, yielding the highest sensitivity for the diagnosis of MMP.

Recommendations

It is recommended that DIF be performed in all patients suspected of having MMP.
Grade of recommendation B

Immunoreactants can also be detected by DIF in non-affected asymptomatic sites.[9,10,12] A recent retrospective study in 251 oral MMP patients compared DIF performed on normal buccal

mucosa with a perilesional punch mucosal biopsy, and detected no significant difference between the two approaches in sensitivity for oral MMP (93.7% vs. 89.6%).[7] Immunoreactants can be detected in a skin biopsy by DIF of affected or non-affected skin (in 44–48%), and may confirm diagnosis of MMP.[2,9,11] A minimum biopsy size of 3–4 mm of skin, and for mucosa, is recommended.[9,13–15] Saline transportation can be used for skin or mucosal biopsies (within 24 h), but is not suitable for conjunctival biopsies.[16,17] Routine testing should be performed for IgG, IgA and complement C3.[2,4,11] IgM and fibrin depositions can also be found in conjunction with other immunoreactants, and as solitary findings in oral lichen planus.[2]

Recommendations

It is recommended that a 3-4 mm punch biopsy be taken for DIF. The biopsy should preferably be taken from perilesional mucosa or skin. If a biopsy of a perilesional location is too painful for the patient, or impractical for the clinician, it can also be obtained from normal mucosa or skin.

Grade of recommendation B

It is recommended to snap-freeze the biopsy, or to use isotonic saline solution (up to 24 hours) or Michel's medium (up to 72 hours) for transportation until processing.

Grade of recommendation C

Negativity of DIF may possibly depend on biopsy site or technical difficulties in cases of conjunctival biopsies, and multiple simultaneous and sequential biopsies may increase the diagnostic yield.[6] Similarly, IgG4 staining may increase the yield of DIF.[18] Positive DIF findings in MMP do not distinguish predominant cutaneous variants of pemphigoid, which should be determined on clinical grounds.[1,12]

Conclusions

Level of evidence 4	Initial negative DIF findings may be due to biopsy site, and/or to a lower amount of tissue-bound autoantibodies in MMP compared to other AIBD, or to technical difficulties in processing mucosal biopsies.

Recommendations

In case of initially negative DIF findings, it is recommended that a sequential biopsy from a different site be performed if clinical suspicion of MMP persists.

Grade of recommendation C

Serration pattern analysis allows for identification of tissue-bound antibodies against type VII collagen.[17,19,20] Serration pattern analysis is often not determinable in mucosal biopsies, but can be performed in biopsies taken from the skin.[17,19,20]

Conclusions

Level of evidence 3	Serration pattern analysis is helpful to identify tissue-bound antibodies against type VII collagen in skin, but often not in mucosal biopsies.

Recommendations

It is recommended that the serration pattern be determined in any skin biopsy for DIF.

Grade of recommendation C

Direct immunoelectron microscopy

Electron microscopy studies allow the analysis of BMZ, including hemidesmosomes, anchoring filaments and anchoring fibrils; these structures cannot be seen by light microscopy. Two techniques of electron microscopy are available: standard transmission electron microscopy and immunoelectron microscopy. Transmission electron microscopy enables precise identification of the level of blister formation, and of structural abnormalities of junction systems which lead to this cleavage. Direct immunoelectron microscopy, like DIF, allows detection of *in vivo* bound IgA, IgG, IgM and/or C3. While DIF gives only linear staining of the BMZ at the dermo-epidermal or chorio-epithelial junction, direct immunoelectron microscopy demonstrates more precise ultrastructural *in vivo* location of antibodies within the dermo-epidermal or chorio-epithelial junctions.

For immunoelectron microscopy, a biopsy must be obtained from clinically normal-appearing skin or mucous membrane adjacent to a lesion within 1–2 cm of the lesions. The minimal diameter size of the biopsy is 6 mm. The sample must be immersed immediately in the appropriate medium[21] and transported within one hour to the laboratory under proper conditions, as any delay will cause it to dry out and result in irreversible damage, making it unsuitable for analysis. Avoid anaesthesia with adrenalin, and taking biopsies of blistered skin, because this often results in artefacts or false-negative results. Details on the detection of binding sites of immune deposits are provided in the Appendix S1.

Conclusions

Level of evidence 4	Direct immunoelectron microscopy is a sensitive and specific assay for detection of tissue-bound IgG and IgA deposits at the dermal-epidermal and/or chorio-epithelial junction.
Level of evidence 4	Direct immunoelectron microscopy enables localization of autoantibodies within the different layers of the basement membrane zone, indirectly reflecting the target antigens, i.e., BP180, laminin 332, and type VII collagen.
Level of evidence 4	The use of direct immunoelectron microscopy is restricted to specialized centers, as the biopsy must be freshly processed and cannot be delivered by mail.

Recommendations

If available, direct immunoelectron microscopy may be recommended for detection of tissue-bound IgG and IgA, in addition to DIF for diagnosis of MMP.
Grade of recommendation C
In particular, direct immunoelectron microscopy may be recommended for precise localization of tissue-bound IgG and IgA in patients with a seronegative MMP.
Grade of recommendation C

Immunoserological tests

Indirect immunofluorescence on tissue substrates IIF detects circulating autoantibodies in the patient's sera through an isotype-specific fluorescent-labelled secondary antibody.[22] In case of MMP, positivity is defined by the detection of linear IgG or IgA along the BMZ of different tissue substrates.[23–30] Compared to bullous pemphigoid, IgG positivity by IIF in MMP occurs less frequently and usually with lower titres.[25,27,31,32] This may be due to the heterogeneity of the target antigens, or the lower amount of autoantibodies in MMP sera. IIF reactivity is largely accounted for by IgG class autoantibodies, but IgA autoantibodies can be detected in about 60% of MMP sera.[27,32,33] Combined IgA and IgG reactivity was associated with more severe disease, compared to the presence IgG autoantibodies alone[32,33] whereas other studies failed to reveal a similar relation.[29,34] One study has reported the presence of circulating anti-BMZ IgE in 24% of 29 MMP patients,[35] but this study awaits confirmation. The sensitivity of IIF depends on the substrate used. Circulating antibodies have been detected in a small percentage of patients on monkey oesophagus substrate, ranging from 2.6% to 8%.[28,29,36] Normal human skin had previously demonstrated higher values (17–35%).[25,31,37] In the salt-split skin technique, normal human or primate skin is incubated in 1 mol/L NaCl until splitting occurs within the lamina lucida of the BMZ.[38] This procedure showed positivity in a significantly greater proportion of MMP patients (36% to 84%).[23,25,27,29,32,39,40] IIF on normal human oral mucosa showed a sensitivity of 85% in a recent study[37], whereas in another study, the same substrate tested negative in all patients.[40] Further investigations are needed to clarify the diagnostic value of oral mucosa as substrate for IIF in MMP. Further details are given in the Appendix S1 and in Table S1.

Conclusions

Level of evidence 3	In MMP, the highest sensitivity of an IIF with a tissue substrate is observed with human/primate salt-split skin.

Level of evidence 3	IgA anti-BMZ reactivity can be found by IIF in about half of serum positive MMP sera on human/primate salt-split skin; IgG anti-BMZ reactivity is detectable in a greater proportion of patients.

Recommendations

It is recommended that IIF on human/primate salt-split skin be used to detect circulating autoantibodies.
Grade of recommendation C
It is recommended that IgG and IgA reactivity be tested by IIF on human/primate salt-split skin.
Grade of recommendation C

Target antigen-specific detection of autoantibodies The target antigens of MMP autoantibodies are components of the epidermal BMZ. Currently, five different target antigens have been identified at the molecular level: BP180 (type XVII collagen), BP230, all three laminin 332 subunits, both subunits of integrin $\alpha6\beta4$ and type VII collagen.[1,41] A pathogenic role of MMP IgG autoantibodies against laminin 332 and $\alpha6\beta4$ integrin has been described.[42–46] Different methods have been established that enable target antigen-specific detection of serum autoantibodies in MMP sera, including ELISA, immunoblotting, immunoprecipitation and indirect IF microscopy (detailed in the Appendix S1). Five assays (ELISA and IIF) applying four target antigens are highly standardized and widely available; they allow the detection of (i) IgG autoantibodies against the 16th non-collagenous domain of BP180 (NC16A), (ii) C- (and N-terminal) part(s) of BP230 and (iii) the laminin 332 heterotrimer. Other serological test systems are available only in specialized laboratories.

It is noteworthy that the reported sensitivities and specificities of these serological tests, discussed in more detail below, are mainly based on studies of selected and well-characterized patients. In addition, some studies have applied cut-off values for these serological tests in MMP that have been established in bullous pemphigoid, e.g. BP180 NC16A- and BP230-specific ELISA.

Detection of antibodies against BP180. BP180 (also termed type XVII collagen) is the most frequent target antigen in MMP and is recognized by approximately 70% of MMP sera.[14,25,27,29,31,33,34,39,47–58] Immunoblotting has been performed using various substrates, including extracts of human cultured keratinocytes from skin and oral mucosa, epidermis or amniotic membrane; keratinocyte hemidesmosome-rich fraction; enriched preparations of the soluble ectodomain of BP180 in medium of cultured keratinocytes (LAD-1); and various recombinant fragments. With these approaches, IgG autoantibodies to BP180 were found in 30–78% of MMP patients, while IgA were detected in 11–51% of MMP sera.[25,27,31,33,34,39,47–54,56,59] In a

cohort of non-scarring oral MMP cases, 75% showed antibodies against BP180.[54] A different cohort of oral MMP cases showed BP180 reactivity in 46% of the cases and found no significant differences in antibody recognition pattern in patients with restricted oral lesions and patients with also other affected sites.[34]

Several studies described the reactivity of MMP sera with the C-terminal portions of the molecule, whether or not combined with a reactivity to the NC16A portion immunodominant in bullous pemphigoid.[1,25,27,33,48,49,52,54,60,61] A large majority of bullous pemphigoid patients also showed reactivity with the C-terminal portion in addition to reactivity with the NC16A domain of BP180.[62] In addition, direct binding of BP180 to type IV collagen, and the capability of antibodies targeting the C-terminus of BP180 to hinder this binding in oral mucosa keratinocytes, has been recently reported.[59] Importantly, anti-BP180 autoAbs are not limited to the IgG isotype, and testing for IgA reactivity increased the detection rate.[25,27,52,61,63] The soluble ectodomain of BP180, originally described as target antigens of linear IgA disease, LAD-1 or a 97 kDa fragment of LAD-1, is recognized by a portion of MMP sera by immunoblotting.[27,33,51,56,60,64,65] Serum levels of autoantibodies to BP180 did not correlate with disease severity in MMP patients.[29,55,66] In line with this, while the pathogenic relevance of IgG antibodies against the NC16A domain is unclear, its murine homologue has been clearly demonstrated in several mouse models, resulting in predominant skin lesions[67–69]; no mouse model has been reported that mimics human MMP based on antibodies against a C-terminal stretch of BP180. However, the presence of autoantibodies recognizing different target antigens, or multiple BP180 epitopes, or belonging to IgG and IgA isotypes, has been observed in MMP patients with more severe clinical features.[33]

Conclusions

Level of evidence 3	BP180 is the major target antigen in MMP.
Level of evidence 3	Anti-BP180 IgG and/or IgA can be found in the majority of seropositive MMP sera.
Level of evidence 3	C-terminal epitopes on BP180 are frequently recognized

Recommendations

It is recommended that IgG antibodies against BP180 NC16A be looked for every patient with no reactivity, or epidermal IgG reactivity, by IIF on human/primate salt-split skin.
Grade of recommendation C
It is recommended that serum IgG and IgA reactivity be tested against BP180 by immunoblotting and/or ELISA.
Grade of recommendation D

Detection of antibodies against BP230 Autoreactivity against BP230 is detected occasionally in MMP sera, with a frequency ranging from 0% to 40%.[25,29,33,34,47–51,54,55,57,64–66,70–72] Anti-

BP230 IgA are absent or less represented than IgG.[34,49] While injection of anti-BP230 antibodies in neonatal mice has resulted in skin lesions, the pathophysiological relevance of antibodies against BP230 in MMP has not yet been demonstrated.[73] In line with this, antibodies against BP230 have not been found to correlate with MMP severity.[29,55,66]

Conclusions

Level of evidence 4	BP230 is targeted by autoantibodies in a minority of MMP patients, usually in conjunction with autoantibodies against BP180 or laminin 332.

Recommendations

It may be considered to search routinely for antibodies against BP230.
Grade of recommendation C

Detection of antibodies against laminin 332 Laminin 332 is the second most frequent target antigen of autoantibodies in MMP.[74] Although IIF on salt-split human/primate skin is a sensitive serological test for detection of circulating autoantibodies in MMP, a portion of MMP sera reactive to laminin 332 are negative when tested by IIF; this emphasizes the relevance of using additional techniques for serological diagnosis.[25,39,55] Until very recently, detection of anti-laminin 332 antibodies was limited to specialized laboratories and performed using different inhouse assays, including immunoblotting, immunoprecipitation and ELISA. After comparison of different methods for the detection of anti-laminin 332 antibodies, immunoprecipitation with radiolabelled keratinocyte extracts was found to be the most sensitive technique, followed by immunoblotting with extracellular matrix of cultured human keratinocytes.[50,75,76] In unselected MMP patients, detection in tested sera of antibodies to laminin 332 by immunoblotting or immunoprecipitation ranged from 4% to 31%.[25,27,29,39,49,50] The α3 subunit of laminin 332 was the most frequently targeted chain, followed by the γ2 subunit,[29,56,71,75–80] and IgG4 was the most strongly represented subclass.[76,80,81] Also serum IgE and IgA were reactive with laminin 332 in small subsets of patients.[56,82]

Several ELISAs for detection of anti-laminin 332 IgG have been established.[55,70,76,81,83] When tested on laminin 332 positive sera from MMP patients, this approach showed high sensitivity but limited specificity, ranging from 75% to 94% and from 60% to 98%, respectively.[70,76,83] In a large group of unselected MMP patients, Bernard and coworkers detected laminin 332 antibodies in 20% of sera, with a specificity of 91% (3/32 of healthy controls).[55] Further, a sensitive (100%, *n* = 16) and specific assay (96.9%, *n* = 127), based on detection by IIF of IgG binding to laminin 332 secreted by human keratinocytes, named the keratinocyte footprint assay, has been reported.[84] Moreover, a sensitive and specific assay based on IIF on HEK293 cells expressing

the laminin 332 heterotrimer on the cell surface (biochip mosaic), has recently been developed. When a large cohort of 93 laminin 332 positive MMP patients was assayed, a sensitivity of 84% and specificity of 99.6% were obtained.[80] This assay is highly standardized and widely available.

Another elegant but non-routine method for the detection of anti-laminin 332 serum antibodies is indirect IF microscopy on the skin of patients with inherited junctional epidermolysis bullosa deficient of laminin 332.[85] However, this method requires reactivity on human/primate skin, and the absence of reactivity with any other BMZ antigen. Furthermore, the availability of laminin 332-deficient skin is limited.

Conclusions

Level of evidence 3	Laminin 332 is the second most frequent target antigen in MMP.
Level of evidence 3	Different assays for the detection of serum antibodies against laminin 332 have been established in specialized laboratories. At present, the indirect IF-based biochip mosaic, with recombinant laminin 332 expressed on the cell surface, is the only assay that is highly standardized and available.
Level of evidence 3	Serum levels of anti-laminin 332 IgG were shown to correlate with disease activity.

Recommendations

It is recommended that patients with MMP be tested for anti-laminin 332 reactivity when indirect IF on salt-split human/primate skin reveals dermal binding, or is negative.

Grade of recommendation B

Detection of antibodies against α6β4 integrin Reactivity of MMP sera with α6β4 integrin was originally described by Ahmed's group.[86] By employing immunoblotting and immunoprecipitation with α6β4 integrin-rich tumour cell lysates (e.g. DU145 prostate cancer cells) and different tissue lysates (bovine and human epidermis, gingiva, conjunctiva), they showed that sera of patients with different clinical phenotypes react specifically with one of the two subunits of the integrin.[87–89] They reported that ocular MMP sera, and MMP sera from patients with at least two involved mucosal sites, recognized the β4 integrin subunit, while oral MMP sera reacted with the α6 integrin subunit.[87,88,90] In addition, anti-β4 and anti-α6 IgG serum levels correlated with disease activity and response to therapy.[66,91,92] A limited number of studies from different laboratories have confirmed the results obtained by Ahmed's group, while other authors failed to detect any α6β4 integrin reactivity in MMP patient sera.[14,54,57] Oyama and coworkers reported that 26 of 124 (21%) of MMP patient sera recognized the β4 integrin subunit; they used immunoblotting on placental amnion proteins, of which 23/26 (88%) had ocular involvement, suggesting that

the β4 integrin might be a site-specific antigenic determinant in MMP with ocular involvement.[33] More recently, analysis of 43 ocular MMP sera by immunoblotting on hemidesmosome-rich fraction showed IgG reactivity to the cytoplasmic domain of β4 integrin in 42% of sera and to the α6 ectodomain in 19%.[56]

Conclusions

Level of evidence 4	Serum antibodies against α6β4 integrin have been detected in a variable proportion of MMP patients in specialized laboratories using in-house assays, and may be site-specific antigenic determinants in MMP with oral (α6 subunit) or ocular (β4 subunit) involvement. Data on their frequency and site-specific associations remain uncertain. Additional confirmative studies by independent laboratories are needed.

Recommendations

No recommendation on the detection of antibodies against α6β4 integrin can be made based on the current data.

Grade of recommendation D

Detection of antibodies against type VII collagen Type VII collagen (Col7) is the major component of anchoring fibrils and the autoantigen of epidermolysis bullosa acquisita (EBA). The serological diagnosis of EBA has previously been discussed in detail in a consensus paper by a group of international experts.[93] Reactivity with Col7 in MMP is rare and may account for fewer than 5% of cases.[29,94] Several assays for the serological detection of anti-Col7 antibodies have been described, including (i) several ELISA systems that apply recombinant forms of Col7; (ii) immunoblotting of recombinant or forms of Col7; (iii) immunoblotting of cell-derived forms of Col7, e.g. in human dermis or an amnion epithelial cell line; (iv) an IIF-based test which uses a human cell line that expresses the recombinant NC1 domain on the cell surface; and (v) indirect IF on Col7-deficient skin.[93] Two of these assays are highly standardized and widely available: an ELISA that employs the recombinant NC1 domain (sensitivity and specificity for EBA, 92.9% and 100%), and an indirect IF-based biochip mosaic, where recombinant NC1 domain is present on the cell surface (sensitivity and specificity for EBA, 87.5% and 100%).[95–99]

Conclusions

Level of evidence 3	Type VII collagen is a rare target antigen in MMP, comprising <5% of cases.
Level of evidence 3	Two test systems for the detection of serum IgG against type VII collagen, an ELISA and an indirect IF-based assay, are highly standardized and widely available.

Recommendations

It is recommended that patients with MMP be tested for anti-type VII collagen reactivity when indirect IF on salt-split human/primate skin reveals dermal binding, or is negative.

Grade of recommendation B

Further details about the detection of autoantibodies against the individual target antigens are provided in the Appendix S1.

Histopathology

Histopathology is less sensitive and specific in diagnosing MMP compared to DIF, and in a recent study reached a sensitivity of 69.4% in 134 patients.[7] Its main role in MMP is to rule out other diseases, e.g. lichen planus, infectious diseases, pemphigus vulgaris and erythema multiforme. The characteristic histopathological picture shows subepithelial splitting, with a non-specific mixed infiltrate consisting of lymphocytes, histiocytes, plasma cells, neutrophils and eosinophils.[7,100–111] However, less eosinophilic granulocytes than in bullous pemphigoid have been observed.[108] Epithelial changes reminiscent of lichen planus with acanthosis, hypergranulosis, as well as vacuolar degeneration with fibrosis and a band-like infiltrate have also been described.[112] However, in many cases, only a non-specific ulcerative inflammation can be seen, with granulation tissue and scarring. In these cases, one cannot differentiate between MMP and aforementioned differential diagnoses. Scarring is commonly seen in late or recurrent lesions. Conjunctival biopsies often lack a subepithelial split, and instead show epithelial metaplasia, a reduced number of goblet cells, fibrosis and a non-specific chronic infiltrate.[15,100,113,114]

Conclusions

Level of evidence 5	A lesional biopsy for histopathology can be useful to differentiate MMP from pemphigus
Level of evidence 5	Histopathology does not differentiate MMP from other pemphigoid disorders, or MMP subgroups from each other.
Level of evidence 5	When MMP is excluded, a lesional biopsy for histopathology can be useful to consider differential diagnoses.

Recommendations

It is recommended to completely include a small intact blister in the biopsy specimen.
If this is not possible, it is recommended to take the biopsy in such a way that it also contains a small amount of perilesional skin (approximately ¼ of the biopsy) to prevent the blister roof from floating off during processing.

Grade of recommendation B

For an oral, pharyngeal, laryngeal, esophageal or genital biopsy, it is recommended to biopsy mucosa directly adjacent to an erosion.

Grade of recommendation B

It is not recommended to biopsy an erosion.

Grade of recommendation B

A standardized 4% formaldehyde (10% formalin) solution is recommended for storage and transport.

Grade of recommendation B

Diagnosis of ocular MMP

Up to 50% of ocular MMP cases do not meet the immunopathological criteria recommended in the 2002 Consensus for a diagnosis of MMP.[1] Because the current standard of care for the causes of cicatrizing conjunctivitis other than MMP is topical therapy, and not the systemic immunomodulatory therapy required for ocular MMP,[36,115,116] implementation of the Consensus guideline has resulted either in delayed diagnosis in individual patients with ocular MMP, or a diagnosis of a non-MMP severe chronic cicatrizing conjunctivitis.[10,117,118] In both situations, inappropriate treatment with topical therapy has resulted in poor outcomes for individual patients. The background to the opposing recommendations regarding the definition and diagnosis of ocular MMP is described in two recent case series, in which 26/55 (47.3%)[117] and 20/73 (27.4%) patients[10] with ocular MMP did not meet current immunopathological criteria for diagnosis, but in whom the clinical phenotype, disease severity and disease course were identical to that in immunopathology positive ocular MMP cases.

The subset of ocular MMP cases with undetectable autoantibodies requires an additional panel of investigations before a diagnosis of ocular MMP can be confirmed, to exclude other cicatrizing conjunctival disorders with a similar disease course. These investigations include both conventional histopathology and a careful clinical history, and systemic examination outlined below.[10,117–121]

1 DIF on the conjunctiva and/or tissue from other sites. Patients with DIF showing IgG, IgA, and/or C3, either in the conjunctiva or from another site, meet the currently widely adopted 2002 Consensus criteria. Biopsy of normal skin or oral mucosa may be positive when a conjunctival biopsy is DIF negative in ocular MMP.[117]

   a Where possible, bulbar conjunctival biopsies should be taken from uninflamed conjunctiva because of the reduced sensitivity in inflamed conjunctiva.[9,122] When they are taken from inflamed conjunctiva, this should be recorded.

   b Biopsies should be taken from another non-lesional site if the conjunctiva is inflamed, and because multiple biopsies improve the detection of a positive DIF.[6] Non-lesional skin gives results similar to those of uninflamed

conjunctiva,[9] and buccal mucosal DIF may also be positive when the conjunctival is negative. More data are needed regarding the numbers of biopsies that are optimal to provide good DIF sensitivity.

   c Conjunctival DIF should also include staining for fibrinogen to identify lichen planus, which shows shaggy discontinuous fibrinogen deposits at the BMZ.[1]

2 Routine conjunctival histopathology is needed to exclude sarcoid and ocular surface tumours, both of which may present with inflammation and scarring. Ocular surface tumours are usually, but not always, unilateral.

3 Serology tests: Patients with positive IIF, or the presence of antibodies to epithelial BMZ proteins, can be diagnosed as having ocular MMP providing the clinical features are consistent. These tests are generally less often positive than DIF[1], and it is important to be aware that a variable proportion of age- and sex-matched healthy controls have positive serology findings (see Table S1).

4 When both DIF and serology are negative, and the other diseases that may cause cicatricial conjunctivitis have been excluded, this immunopathology negative subset of patients can be diagnosed as having ocular MMP. However, if the disease course or response to therapy is not as expected, all tests should be repeated.

Conclusions

Level of evidence 3	Ideally, bulbar conjunctival biopsies are taken from uninflamed conjunctiva, where possible, because of the reduced sensitivity in inflamed conjunctiva.
Level of evidence 3	Non-lesional skin gives similar results to uninflamed conjunctiva, and buccal mucosal DIF may also be positive when the conjunctival is negative.
Level of evidence 3	Ocular surface tumors are usually, but not always, unilateral.

Recommendations

In ocular MMP, it is recommended that the following investigations be performed: DIF of non-inflamed conjunctiva and buccal mucosal biopsies; histopathology of a lesional (inflamed and thickened, but not ulcerated) conjunctival biopsy; and serology. When DIF biopsies are taken from inflamed conjunctiva this should be recorded.
Grade of recommendation D
Conjunctival DIF should also include staining for fibrinogen, as shaggy discontinuous deposits at the BMZ are suggestive of lichen planus.
Grade of recommendation D

If uninflamed conjunctiva is not available for biopsy it is recommended to proceed with a biopsy as outlined in the section on *DIF*.
Grade of recommendation C
If the initial DIF is negative it is recommended to proceed with a biopsy as outlined in the section on *DIF*.
Grade of recommendation D
Routine conjunctival histopathology is recommended to exclude sarcoid and ocular surface tumors, both of which may present with inflammation and scarring.
Grade of recommendation D
It is recommended that subjects with circulating antibodies to epithelial BMZ proteins be diagnosed as having ocular MMP, providing the clinical features are consistent. Serological tests are generally less often positive than DIF.
Grade of recommendation D

Algorithm for the diagnosis of MMP

The recommended algorithm for the diagnosis of MMP is shown in Fig. 1.

Differential diagnoses of MMP

When repeated DIF and serology are negative, the diagnosis of MMP cannot be made, with the exception of individual cases of ocular MMP. In these rare cases, differential diagnoses need to be considered by an experienced ophthalmologist.

If multiple sites are involved, in particular the eyes, only few differential diagnoses remain, including pemphigus vulgaris (intraepithelial splitting by histopathology, antibodies against desmoglein 3, intercellular binding of autoantibodies in the epithelium by DIF), erythema multiforme, Steven Johnson syndrome and toxic epidermal necrolysis.

In single-site MMP, the following differential diagnoses may be addressed:

Oral MMP: Herpes simplex virus infection, *Candida* infection, lichen planus, aphthous stomatitis, systemic lupus erythematosus, erythema multiforme, Steven Johnson syndrome, toxic epidermal necrolysis, leukoplakia, Crohn's disease, malnutrition, radiation mucositis and chemotherapy-induced mucositis.

Ocular MMP: Rosacea, viral and bacterial infections, atopic keratoconjunctivitis, trauma, malignant tumours, Sjögren's syndrome, systemic lupus erythematosus, sarcoidosis.

Genital MMP: Lichen sclerosus et atrophicus, erosive lichen planus, pemphigus and sexual abuse.

Laryngeal MMP: Pemphigus, epidermolysis bullosa and malignancy.

Conclusions

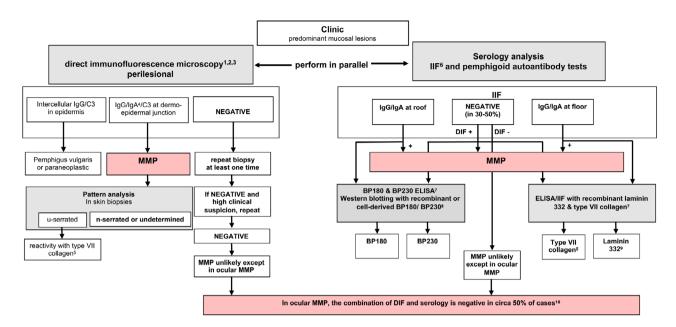| Level of evidence 3	In case MMP cannot be diagnosed according to the diagnostic algorithm for MMP, a number of differential diagnoses need to be addressed.

Figure 1 Diagnostic algorithm and work-up, and diagnostic criteria for mucous membrane pemphigoid. [1]Alternatively or in addition, direct immunoelectron microcopy can be performed. [2]A positive DIF from any site is diagnostic for MMP, providing the clinical phenotype at the site that has not been biopsied is consistent with MMP. [3]If ocular MMP is suspected, take biopsies from the least inflamed bulbar conjunctiva of both eyes together with another site (buccal mucosa or skin). Also take an additional lesional biopsy for routine histo-pathology to exclude both ocular surface neoplasia and sarcoid (which may present in the conjunctiva). [4]Patients with predominant or exclusive IgA deposition could also be classified as having linear IgA disease. [5]Patients with reactivity with type VII collagen could also be classified as having Epidermolysis Bullosa Acquisita. [6]On human/primate salt-split skin. [7]Commercially available (for IgG antibodies). [8]Only available in specialized diagnostic centers. [9]Associated with a malignancy in 25–30% of patients; a tumor search is indicated. [10]A diagnosis of immunopathology unconfirmed ocular monosite MMP can be made by exclusion of the more than 25 other causes of cica-trising conjunctivitis (CC). MMP is the most common cause of CC in most developed countries. Causes of CC, except for sarcoid & sur-face neoplasia: (i) have a history consistent with another cause of conjunctival disease; (ii) are positive on routine histopathology for neoplasia or sarcoid; or (iii) are DIF+ for another immuno-bullous disease. If, after initiating appropriate therapy for immunopathology negative ocular monosite MMP, the disease course or response to therapy is not as expected, then this algorithm (both for DIF in ocular cases and serology) should be repeated and alternative diagnoses considered (e.g., severe ocular rosacea which can be difficult to differ-entiate from ocular MMP). DIF, direct immunofluorescence microscopy; ELISA, enzyme-linked immuno sorbent assay; IIF, indirect immunofluorescence microscopy; MMP, mucous membrane pemphigoid.

Recommendations

In case MMP cannot be diagnosed according to the diagnostic algo-rithm for MMP, it is recommended that the following interventions be performed to address major differential diagnoses:

- detection of serum autoantibodies against desmoglein (for pemphigus vulgaris)
- review of lesional biopsy (for toxic epidermal necrolysis, lichen planus, etc.)
- swabbing for *Herpes simplex* virus infection
- swabbing for *Candida* infection

Grade of recommendation D

Management

Aim of therapy and multidisciplinary care

The aim of treatment is to stop the inflammation, and hereby the progression of scarring, especially of conjunctivae, larynx,

oesophagus and genital mucous membranes. Surgical release of scarring and strictures is indicated only after the inflammatory phase of MMP has been fully controlled for several months.

Management of MMP requires a multidisciplinary team, involving specialists from dermatology, ophthalmology, otorhi-nolaryngology, gastroenterology and gynaecology/urology. Sys-temic treatment is ideally co-ordinated by a specialist, collaborating with the clinicians treating the complications of disease at other sites of involvement. In centres focusing on the management of MMP, often an established multidisciplinary team (dermatologist, ophthalmologist, stomatologist, otorhino-laryngology, etc.) is involved in the diagnostics and follow-up of the patients, including management of complications, and depending on the affected sites.

Mild MMP (moderate) and severe MMP are defined accord-ing to Chan *et al.*: patients with mild MMP have disease occur-ring only in oral mucosa, or in oral mucosa and skin. Patients

with severe MMP have disease occurring in any of the following sites: ocular, genital, nasopharyngeal, oesophageal and/or laryngeal mucosae.[1]

Conclusion

Level of evidence 5	The aim of treatment in MMP is to stop inflammation, and hereby stop progression of scarring, especially of eyes, larynx, esophagus, and genital mucous membranes.

Recommendations

It is recommended that newly diagnosed MMP patients be screened by an ophthalmologist, an oral medicine specialist or an experienced dermatologists and an otorhinolaryngologist at baseline, and during follow-up in case of clinical symptoms.

Grade of recommendation D

Topical medications

Oral MMP Topical therapies available for use in oral MMP include a broad range of corticosteroids, or the calcineurin inhibitor tacrolimus. There are no randomized placebo-controlled trials to support efficacy of topical therapies in MMP. Evidence is based largely upon small case series or RCTs conducted to study mixed oral vesiculoerosive disease. Nevertheless, the findings of these studies are frequently used in clinical practice.

Topical corticosteroid therapy was advocated in the First Consensus statement on MMP[1] for mild to moderate MMP as a first-line approach, and more recently, the available evidence was evaluated in a systematic review.[123] This therapy is often used in clinical practice for mild or moderate disease oral MMP as first-line therapy, and in more severe disease, it is used in addition to systemic therapy for patients with multisite or single-site disease. Topical steroids, particularly the superpotent clobetasol propionate, can lead to remission.[124,125] The latter corticosteroid is the most frequently used topical ointment, while betamethasone sodium phosphate tablets 0.5mg may be diluted in water and used for rinsing for 2–3 min before discarding, between one and four times per day. Fluticasone propionate 400 micrograms (1 mg/mL) may also be used twice daily as a mouthwash. Corticosteroid metered-dosed inhalers may be sprayed directly onto active lesions. The frequency of use is tailored to the severity of the disease, with one application ideally before sleep, as saliva flow is reduced overnight and the length of contact is therefore optimized; applications can be tapered as lesions improve.

For gingival lesions, use of a custom-made, soft drug-delivery tray covering the gingivae to extend drug contact time and absorption, has been described.[126] This is a method sometimes used in routine clinical practice, though no study has compared its efficacy with other methods of application. Adjuvant analgesic, anti-inflammatory and anti-infectious therapy can be additionally used, e.g. chlorhexidine 0.12–0.20%.

There are case reports demonstrating efficacy of topical tacrolimus in localized oral MMP, and reporting complete remission within 2–3 months. However, the cost is greater, and tolerance may be lower due to oral burning upon application.[127–129] No good evidence supports the use of topical cyclosporine for oral MMP. Further details on the topical treatment of oral MMP are provided in the Appendix S1.

Conclusions

Level of evidence 4	Evidence for use of topical therapy in MMP is limited to small case series or RCTs conducted in mixed oral vesiculoerosive diseases. Evidence supports the use of topical corticosteroids for MMP.

Recommendations

Topical corticosteroids can be recommended as first-line therapy in mild/moderate MMP, and as adjunctive therapy in moderate to severe oral MMP.

Grade of recommendation D

Ocular MMP Historical evidence suggests that topical therapy does not alter the natural history of the disease, and offers only variable symptomatic relief.[130–133] But in patients intolerant to immunosuppression, or where it is not safe to administer immunosuppression, then topical steroids, combined with systemic matrix-metallo proteinase inhibitors (tetracyclines), are a useful alternative for treating mild disease. Subconjunctival steroids, such as triamcinolone, may provide temporary benefit, but relapses may occur, together with complications such as cataract, glaucoma or localized scleral thinning. Topical tacrolimus and ciclosporin have been used in isolated cases with limited response.[134–137] Topical treatment in the form of lubricant drops, gels and ointments should be used to reduce trauma. These lubricants should preferably be free of preservatives to avoid iatrogenic toxicity. Serum eye drops may be used as alternative, or in addition, to provide nutrients to severely dry ocular surfaces.

Conclusions

Level of evidence 4	Topical therapies may offer symptom relief, but do not influence immune-mediated disease course.
Level of evidence 4	Subconjunctival corticosteroids, such as triamcinolone, may provide temporary benefit, but relapses may occur, together with complications such as cataract, glaucoma, or localized scleral thinning. Topical tacrolimus and ciclosporin have been used in isolated cases with limited response.

Recommendations

Use of topical steroids may be recommended as an ancillary short-term treatment for ocular involvement. Topical cyclosporine may be considered as an adjunct. Other topical treatments in the form of lubricants are recommended to reduce trauma. Serum eye drops can substitute the nutrient effect of tears in severe dry eye.

Grade of recommendation C

In cases of intolerance of immunosuppressive drugs, topical therapies combined with systemic tetracyclines may be recommended as a useful alternative for mildly inflamed ocular disease.

Grade of recommendation D

Genital MMP Only case reports on genital MMP have been presented in the literature. Topical corticosteroids, particularly clobetasol propionate, can lead to remission in juvenile MMP.[138,139] Topical tacrolimus was reported to be effective as monotherapy in one case report of juvenile MMP.[140] In two case reports, topical corticosteroids were ineffective in controlling the progression of juvenile MMP, and dapsone was added.[141,142] Farrell *et al.* reported remission of three patients with juvenile genital MMP treated with topical clobetasol propionate cream and tetracycline combined cream. Two patients required systemic corticosteroids, sulphones, azathioprine and dapsone.[143] Topical therapy in adult MMP is often not sufficient to achieve remission of genital lesions.[144,145]

Recommendations

In mild/moderate genital MMP, high-potency topical corticosteroids alone may be considered as first-line therapy.

Grade of recommendation C

Systemic medications

Disease control has previously been defined as the point at which new inflammatory lesions cease to form and established lesions begin to heal.[146] Immunosuppressive agents need to be chosen with a 'stepladder' approach, beginning with drugs that have the fewest side effects.

Recommendations

It is recommended that patients be defined as refractory when no disease control has been achieved after 12 weeks of adequately administered therapy.

Grade of recommendation D

Tetracyclines Tetracyclines are generally used as antibiotics due to their efficacy in controlling bacterial proliferation and growth. In addition to these effects, tetracyclines have been shown to have also anti-inflammatory and collagenolytic properties. In light of their anti-inflammatory action, tetracycline has

been proposed as first-line agent in mild/moderate MMP, also due to its better side-effect profile as compared to corticosteroids and other conventional immunosuppressive agents.[147,148] Most patients included in the studies taken into consideration have been switched to tetracyclines due to adverse effects with previous treatments. On the other hand, minocycline has been stopped in five out of nine patients included in a case series of predominantly oral MMP due to its side effects, mainly vertigo and gastric upset.[149] In this case series with a follow-up of 2 years, only one patient achieved persistent remission with no relapse.

Conclusion(s)

Level of evidence 4	Tetracyclines, in particular tetracycline (possibly associated with oral nicotinamide), may be effective in the treatment of mild/moderate MMP, with less side effects compared with corticosteroids.

Recommendations

Tetracyclines, i.e tetracycline 1,500 mg/day, may be considered as a first-line treatment in mild/moderate MMP. In refractory cases, oral corticosteroids, mycophenolate or azathioprine may be added.

Grade of recommendation C

Dapsone Dapsone, a well-known anti-leprosy drug, is effective in several dermatologic diseases due to its anti-inflammatory properties. The corticosteroid-sparing effect of dapsone could be explained by several mechanisms, including oxygen-radical scavenging, reduction in tumour necrosis factor (TNF)-α and dysregulation of lymphocyte function. Dapsone is used to treat both mild/moderate and severe cases of different autoimmune bullous diseases, usually in association with corticosteroids. Prior to initiation of therapy, the patient's glucose-6-phosphate dehydrogenase (G6PD) level should be checked to be normal, since low levels are associated with a higher incidence of haemolytic anaemia.

Due to its anti-inflammatory properties, dapsone is regarded as a first-choice treatment for mild/moderate MMP. In a cross-sectional retrospective review, seven out of 20 patients with oral MMP were maintained successfully on dapsone 50–150 mg/day.[150] However, possible side effects caused by dapsone: haemolytic anaemia, skin rash, malaise and gastrointestinal problems, have led to high discontinuation rates in different trials.[151–154]

Conclusions

Level of evidence 4	Dapsone may lead to disease control in mild/moderate MMP. However, adverse effects are quite common. Confirmation of the G6PD status prior to dapsone initiation is necessary.

Recommendations

Dapsone, at a dosage of 1-1.5 mg/kg/day, alone or in combination with topical corticosteroids, may be recommended as first-line treatment for mild/moderate MMP. Careful monitoring of possible onset of side effects is required.

Grade of recommendation C

In refractory cases, oral corticosteroids, mycophenolate mofetil, or azathioprine may be added.

Grade of recommendation C

In severe MMP, dapsone in combination with oral corticosteroids or cyclophosphamide may be considered as a first-line treatment.

Grade of recommendation C

In refractory cases, rituximab (first step), intravenous immunoglobulins (second step), or a TNF-alpha inhibitor (third step) can be added.

Grade of recommendation C

Mycophenolate mofetil Mycophenolate mofetil (MMF) is a prodrug of mycophenolic acid and inhibits the *de novo* pathway of guanosine nucleotide synthesis. T- and B-lymphocytes are critically dependent on this pathway for their proliferation, but the potent cytostatic effects of MMF inhibit proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation. Mycophenolic acid also suppresses antibody formation by B-lymphocytes. The use of MMF for the treatment of mild/moderate or severe MMP has been investigated in few clinical trials.[153,155–157] Its efficacy in controlling inflammatory lesions and its safety, either in monotherapy or in association with corticosteroids, have been confirmed in all these studies.

Conclusions

Level of evidence 4	MMF is an effective agent for treatment of mild/moderate MMP, with minimal side effects. However, the drug cannot always prevent disease progression in severe refractory cases.

Recommendations

MMF, at a dosage of 2 g/day, alone or in combination with topical/oral corticosteroids, tetracycline or dapsone, may be recommended as second-line therapy in patients with mild/moderate MMP.

Grade of recommendation C

Cyclophosphamide Cyclophosphamide is an oxazaphosphorine-substituted nitrogen mustard alkylating agent, with powerful cytotoxic and immunosuppressive effects. It is used to treat haematological and solid cancers as well as autoimmune diseases, including refractory and/or severe autoimmune bullous diseases. Main side effects of cyclophosphamide are haemorrhagic cystitis, infertility and bladder cancer.

Different clinical studies on cyclophosphamide, administered orally or intravenously, demonstrated its effectiveness in severe MMP,[158–163] and it has been shown to be effective for many years.[164] Both oral and intravenous pulsed cyclophosphamide showed a high rate of efficacy, preventing relapses in ocular MMP and allowing to taper corticosteroids. It induced sustained clinical remission both as monotherapy[160] and in combination with corticosteroids[158,161,163] or pentoxyfilline.[163]

Conclusions

Level of evidence 4	Patients with severe MMP, particularly with ocular presentation, have rapidly benefited from cyclophosphamide, also experiencing prolonged remissions. Early initiation of therapy could decrease the risk of relapses.

Recommendations

Cyclophosphamide, administered either orally at an initial dosage of 2 mg/kg/day or intravenously at a pulsed dosage of 500 mg monthly, may be recommended as first-line treatment in severe MMP, either alone or in combination with oral corticosteroids or dapsone.

Pentoxyfylline may be added to the treatment with cyclophosphamide plus corticosteroids in patients with severe MMP.

Grade of recommendation C

Corticosteroids Systemic corticosteroids are widely used for their excellent anti-inflammatory and immunosuppressive effects. A wide range of dermatoses, including autoimmune bullous diseases, are successfully treated with systemic steroids. However, the chronic courses of treatment required favour the onset of side effects, such as osteoporosis, adrenal suppression, hyperglycaemia, dyslipidaemia, cardiovascular disease, Cushing's syndrome and psychiatric disturbances. Steroids may be administered orally, intravenously or through intramuscular injections.

Although systemic corticosteroids (initial oral prednisone 0.5–1.5 mg/kg/day) are effective in achieving rapid control in cases of acute, severe disease, the adverse effects associated with long-term use limit their value. Systemic corticosteroids are usually associated in combination with MMF as second-line treatment in mild/moderate MMP[153,157] and in combination with cyclophosphamide in severe MMP.[162,163] The use of systemic corticosteroids has also been investigated in combination with rituximab.[165] Studies focusing on systemic corticosteroids in monotherapy have not been found, but in clinical practice, they are widely used, even at high dosages, for controlling flare-ups.

Conclusions

Level of evidence 4	Corticosteroids are useful adjuvant agents in both mild/moderate and severe cases. Their side effects limit a prolonged use.

Recommendations

> Oral corticosteroids, i.e., prednisone at an initial dosage of 0.5-1 mg/kg/day tapering over the next 8-12 weeks, may be considered in combination with a corticosteroid-sparing immunosuppressive agent.
>
> **Grade of recommendation C**

Methotrexate Methotrexate is an antifolic and antimetabolic drug widely used for autoimmune and haematological diseases. It is used in dermatology as a steroid-sparing immunomodulating agent. Its mechanism of action is based on its interference with DNA synthesis and replication, as well as the inhibition of rapidly dividing cells.

McCluskey *et al.* reported that an approximately 15-month course of methotrexate therapy led to complete control and/or suppression of conjunctival inflammation in 10 out of 12 (83%) patients with ocular MMP.[166] Moreover, only 24% of patients developed side effects requiring cessation of methotrexate therapy, and these were reversible. In a retrospective, non-controlled, case series study involving 11 patients with severe ocular MMP, Shi *et al.* demonstrated that low-dose methotrexate improved visual acuity in three patients.[167]

Conclusions

Level of evidence 4	Methotrexate monotherapy may be considered as first-line systemic treatment of ocular MMP.

Recommendations

> Methotrexate, at an initial dosage of 7-15 mg/week, alone or in combination with topical corticosteroids, may be considered as first-line treatment in patients with mild/moderate MMP. In refractory cases, oral corticosteroids may be added.
>
> **Grade of recommendation C**

Azathioprine Azathioprine is a synthetic purine analog derived from 6-mercaptopurine, which is thought to act by disrupting nucleic acid synthesis, and has recently been found to interfere with T-cell activation. Albeit originally developed for its anti-cancer properties, azathioprine is nowadays more widely used for its immunosuppressant properties. One of the most recognized uses of azathioprine in dermatology is as treatment for autoimmune bullous disorders, including MMP.

Azathioprine showed a low success rate as compared to methotrexate and dapsone, and its discontinuation due to adverse effects (gastrointestinal, headache, malaise, dizziness, elevated liver function tests and myelosuppression) was higher than in patients treated with other immunosuppressants. In fact, successful treatment was achieved in 43% and 47% of MMP patients treated with azathioprine by Pasadhika *et al.* and Saw *et al.*, respectively.[153,168] In MMP with ocular involvement, an

evaluation of 115 patients on a variety of therapies found that azathioprine had a success rate (no conjunctival inflammation) of 38/80 (47%) and qualified success (partial inflammation control) in 19/80 (24%), with failure in 23/80 (29%). However, the side-effect profile was poor, resulting in discontinuations in 24/60 (40%). For the latter reason, mycophenolate was recommended for use in this study, instead of azathioprine (except as a second-line agent for patients not tolerating mycophenolate), because of the higher success rate in 27/46 (59%) and improved tolerance, resulting in discontinuations of 15% (5/34).[153]

Conclusions

Level of evidence 4	Successful treatment of MMP was achieved in around 50% of MMP patients treated with azathioprine, but with a poorer side effect profile than the other drugs used in this study (dapsone, sulfapyridine, mycophenolate and cyclophosphamide).

Recommendations

> Azathioprine, at an initial dosage of 1.5-2 mg/kg/day, in combination with topical corticosteroids, tetracyclines or dapsone, may be considered as a second-line therapy in mild/moderate MMP.
>
> **Grade of recommendation C**

Rituximab Rituximab is a chimeric monoclonal antibody directed against CD20, which is a cell surface marker expressed by B cells. Recently licensed for moderate/severe pemphigus,[169] it has a long record in refractory autoimmune blistering diseases, including MMP.[170–172] Le-Roux-Villet *et al.* found that 17 out of the 25 patients with severe refractory MMP included in their study showed a complete response after one cycle, and five additional patients after a second cycle, yielding a 88% response rate. Three patients (all receiving concomitant high-dose immunosuppressants) developed severe infectious complications.[173] In this study, immunosuppressant regimens were discontinued at the initiation of rituximab and 88% of patients were continued on maintenance dapsone or sulfasalazine. You *et al.* found that 46 eyes (77.0%) in 26 MMP patients treated with rituximab alone, or in combination with other immunomodulatory treatments, achieved clinical remission, with an average sustained remission time of 24.5 months.[174]

Maley *et al.* studied 24 patients treated with rituximab added to conventional immunosuppression, and 25 treated only with conventional immunosuppression. They found that 100% of patients in the rituximab group achieved disease control compared with 40% in the conventional group ($P < 0.01$), with a mean time to disease control of 10.17 and 37.7 months ($P = 0.02$).[165] Recently, Lamberts *et al.* observed in a cohort of 14 MMP patients treated with rituximab 1 g at day 1 and at day 14, disease control in 85.7%, partial response in 64.3%, and complete response in 28.6% patients, with a relapse rate of 75%

during follow-up.[175] Since 2018, an open-label, phase 3 clinical trial comparing the safety and effectiveness of RTX vs oral cyclophosphamide is ongoing (NCT: 03295383).

Conclusions

Level of evidence 4	Rituximab, alone or combined with other immunosuppressants or intravenous immunoglobulins, is effective in patients with severe, refractory MMP. The onset of adverse events, particularly severe infections, is a common concern in patients treated with rituximab.

Recommendations

Rituximab, either at an initial dose regimen of 375 mg/m² each week for 4 consecutive weeks, or of 1 g given 15 days apart, may be recommended as a second-line treatment in severe MMP, and as third-line treatment in mild/moderate MMP refractory to conventional immunosuppressants.

Grade of recommendation C

Intravenous human immunoglobulins   Intravenous immunoglobulins (IVIg) are a purified IgG preparation derived from pooled human plasma, and contain more than 95% of unmodified IgG, which has functionally intact Fc-dependent effector functions. IVIg may be a therapeutic option in several dermatologic diseases, including autoimmune bullous diseases, and is usually applied at a dose of 2 g/kg body weight over 2–4 days at monthly intervals.[176] IVIg are used when conventional therapies are contraindicated, or when the disease is progressive despite conventional systemic therapies. Adverse events are usually mild, self-limiting and apparently predominantly infusion-related. The most frequent are headache, back pain, chills, flushing, fever, hypertension, myalgia, nausea and vomiting. The major limitation of IVIg is their cost.

IVIg have been reported as an effective and safe treatment for progressive MMP unresponsive to conventional therapies. Letko et al.[177] compared two groups of ocular MMP – one treated with IVIg and the second with conventional therapies – revealing a faster, more effective, and safer response in the first group. Leuci et al.[178] showed that the efficacy of IVIGs is persistent for a long time. Foster et al. and Steger et al. confirmed the favourable response of severe and progressive ocular MMP to combination therapy with rituximab and IVIg.[179,180] Despite evidence of progressive scarring in some of their patients, blindness was prevented. Adverse events reported were limited (headache and nausea), and the authors did not induce discontinuation of the therapy in almost all but two patients described by Segura et al.[181]

Conclusions

Level of evidence 4	IVIGs are effective and safe for severe MMP. Their good safety profile makes them a favorable option for immunocompromised patients who cannot be treated with conventional immunosuppressive regimens.

Recommendations

IVIGs may be recommended as third-line treatment for severe MMP, unresponsive to conventional immunosuppressants and/or rituximab. It is also recommended when a patient is at high risk of developing adverse events to conventional therapies.
Grade of recommendation C

Anti-TNFα drugs   Increased levels of TNFα have been observed in the sera of MMP patients, compared with controls. The use of anti-TNFα drugs in MMP is supported only by case reports or case series, such as that by Canizares et al. reporting on the effectiveness of etanercept in three patients with ocular MMP.[182] Etanercept is a recombinant human dimeric fusion protein consisting of the extracellular ligand-binding domain of the TNFα receptor fused to the Fc portion of the human IgG1.

Conclusions

Level of evidence 5	Controlled trials are needed to confirm the effectiveness and safety of anti-TNFα drugs for MMP.

Recommendations

TNFα inhibitors may be considered as fourth-line therapy for severe MMP.
Grade of recommendation C

Algorithm for the treatment of MMP

The recommended algorithm for the treatment of MMP is shown in Fig. 2.

Systemic medical management of ocular, oral, genital, laryngeal and oesophageal MMP

Ocular MMP

Conclusions

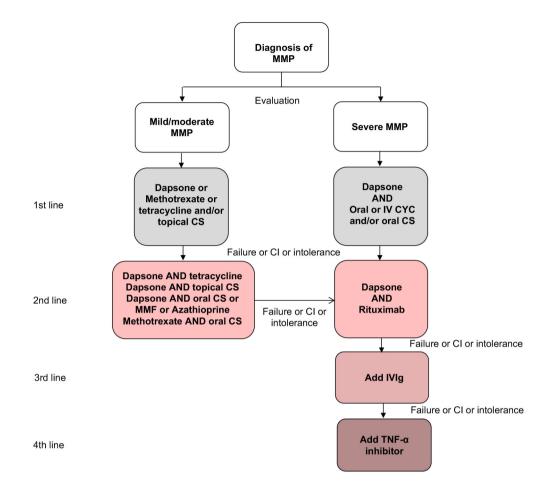| Level of evidence 4	Systemic oral corticosteroids (prednisolone), with or without supplementary loading doses of 1g intravenous corticosteroids (methylprednisolone) preceding oral therapy, tapering over 3 months until adjunctive immunosuppressive agents take full effect, are useful for moderate and severe inflammation in ocular MMP.
Level of evidence 4	Biological therapies such as anti-CD20 (e.g., rituximab) or anti-TNF-α may be beneficial in patients resistant to conventional immunosuppression for moderate and severe inflammation in ocular MMP.
Level of evidence 4	IVIg can be used, either alone or in combination with anti-CD20 therapy, in severe sight-threatening disease or resistant cases of ocular MMP.
Level of evidence 3	Good control of inflammation with systemic immunosuppression is required to minimize progression of conjunctival scarring. Inflammation progresses without visible inflammation.
Level of evidence 4	Subconjunctival mitomycin has been described to reduce fibrosis with mixed results in fewer than 15 subjects reported before 2004.

Figure 2 Algorithm for treatment of mucous membrane pemphigoid. CI, contraindication; CS, corticosteroid; CYC, cyclophosphamide; IV, intravenous; IVIg, Intravenous Immunoglobulin; MMF, mycophenolate mofetil; MMP, mucous membrane pemphigoid; TNF, tumour necrosis factor.

Recommendations

Systemic therapy in ocular MMP is recommended according to the stepladder approach detailed in **Fig. 3**.
Grade of recommendation D
Good control of inflammation with immunosuppression is required to limit progression of conjunctival scarring.
Grade of recommendation B

Oral MMP
Conclusions

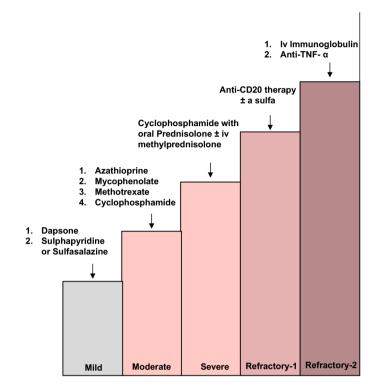| Level of evidence 4	Topical treatment, in particular clobetasol propionate ointment in adhesive paste, is a first-line option in mild/moderate oral MMP. Dapsone, possibly associated with oral or topical corticosteroids, is a first-line agent in severe oral MMP. Combination of systemic corticosteroids, dapsone and immunosuppressive agents, notably mycophenolate mofetil, should be reserved for severe cases.

Figure 3 Algorithm for systemic treatment of ocular mucous membrane pemphigoid. The complete legend is shown in the Appendix S1.

Recommendations

In mild/moderate MMP, oral corticosteroids in combination with dapsone may be recommended as first-line regimen.
Grade of recommendation C
High-dose oral tetracyclines may be considered as second-line agents.
Grade of recommendation C
Combination of systemic corticosteroids, dapsone, and immunosuppressive agents, notably mycophenolate mofetil, may be recommended for severe cases.
Grade of recommendation C

See also above recommendations for topical treatment in ocular disease.

Laryngeal MMP
Conclusions

Level of evidence 4	Dapsone should be the first therapeutic option in mild/moderate laryngeal MMP, while prednisone plus cyclophosphamide should be initiated in unresponsive cases. In severe laryngeal MMP, high dose prednisone combined with cyclophosphamide (or azathioprine) should be regarded as first-line treatment. Rituximab may be considered in severe laryngeal MMP refractory to traditional immunosuppressants. Surgical approach by endoscopic CO_2 laser and dilatation is useful to maintain laryngeal airway, but should be avoided during active phase of the disease.

Recommendations

Oesophageal MMP

In severe laryngeal involvement of MMP, high-dose oral corticosteroids in combination with cyclophosphamide or azathioprine may be considered the first-line regimens. In severe cases, rituximab may be recommended as a second-line treatment option.
Grade of recommendation C
In mild/moderate laryngeal involvement of MMP, dapsone may be considered.
Grade of recommendation C
In laryngeal involvement of MMP, surgical treatment by CO_2 laser excision or dilatation is recommended to treat scarring, but is not recommended during the active phase of the disease.
Grade of recommendation C

Conclusions

Level of evidence 4	Dapsone, cyclophosphamide, and azathioprine can be used to treat esophageal mucous membrane pemphigoid.

Recommendations

In mild/moderate esophageal MMP involvement, dapsone may be recommended as first-line treatment; prednisone plus cyclophosphamide may be considered as second-line treatment.
Grade of recommendation C
In severe esophageal MMP involvement, high-dose oral corticosteroids in combination with cyclophosphamide or azathioprine may be considered the first-line regimen.
Grade of recommendation C
Endoscopic dilatation may be recommended, and should be done by expert operators, to treat cicatricial stenosis upon obtaining disease control by using medical treatment.
Grade of recommendation C

Genital MMP
Conclusions

Level of evidence 4	Dapsone, cyclophosphamide, and azathioprine can be used in the treatment of genital mucous membrane pemphigoid..

Recommendations

In mild/moderate genital MMP involvement, dapsone may be recommended, with or without topical corticosteroids (classes III and IV).
Grade of recommendation C
In severe or refractory genital involvement of MMP, high-dose oral corticosteroids in combination with cyclophosphamide, or azathioprine, or mycophenolate mofetil may be considered. In severe or refractory genital involvement of MMP, high-dose oral corticosteroids in combination with cyclophosphamide, or azathioprine, or mycophenolate mofetil may be considered.
Grade of recommendation C

See also above recommendations for topical treatment in genital disease.

Details on systemic treatment of single-site MMP are shown in the Appendix S1.

Rescue procedures in ocular involvement

Conclusions

Level of evidence 4	Potent immunosuppression cover is required for surgical procedures that breach the conjunctiva, to provide prophylaxis against severe progression of ocular MMP.
Level of evidence 3	In cases of stem cell failure, amniotic membrane grafting, corneal limbal stem cell allograft, and cultured oral mucosal epithelial cells may be considered.
Level of evidence 3	Keratoprosthesis surgery is high risk. Good visual outcomes can be achieved with the osteo-odonto keratoprosthesis for bilaterally blind patients.

Recommendations

For incisional conjunctival surgery, potent immunosuppression is recommended.
Grade of recommendation B
Ocular reconstructive surgery with stem cell replacement surgery may be considered in the bilaterally blind.
Grade of recommendation B
Osteo-odonto keratoprosthesis may be beneficial for patients with bilateral corneal blindness caused by MMP.
Grade of recommendation B

Details on rescue procedures and additional local measures in ocular involvement in MMP are shown in the Appendix S1.

Oral hygiene advice

MMP, among other disorders causing desquamative gingivitis, may potentially intensify the development and progression of plaque-related periodontal disease. A number of studies have described the gingival status in patients with MMP.[183–186] A systematic review showed an increased incidence of periodontitis in patients with desquamative gingivitis (MMP, $n = 65$) compared to healthy individuals.[187] This review showed that patients had worse periodontal parameters, including bleeding upon probing clinical attachment level of the periodontal ligament, probing depth, plaque index and/or gingival index/recession. Patients with a diagnosis of MMP >5 years were also shown to have more recession and furcation involvement.[183] Desquamative gingivitis may indirectly increase the long-term risk for developing periodontal disease via plaque accumulation when pain associated with such lesions impairs capacity to perform efficient oral hygiene practices. In addition, discomfort associated with

gingival lesions could predispose patients to less frequent dental visits. A direct effect of MMP on periodontitis may also be plausible based on the possible shared pathogenic mechanisms between antibody and bacterial-induced inflammatory tissue damage.

Improving oral hygiene is prudent, as this may reduce the chronicity of the disease and the need for complex treatments. In conjunction with medical therapy, the avoidance of trauma and elimination of infection is beneficial. There is evidence for the beneficial effect of conservative treatment in improving the clinical parameters and severity of MMP lesions or symptoms. Non-surgical periodontal therapy, consisting of scaling and root planing, and effective bacterial plaque control can be effective in reducing the gingival manifestations, representing a complementary treatment to the use of corticosteroids.[188–192] A recent systematic review evaluated the efficacy of daily hygiene and professional prophylaxis for treatment of desquamative gingivitis, regardless of its aetiology.[192] This review concluded that the combination of appropriate daily gingival hygiene techniques at home, and the performance of periodontal treatment, including scaling and root planning, decreased pain-perception, disease activity, dental plaque and gingival bleeding. General dentists, hygienists and periodontists therefore play a key role in controlling the oral manifestations of MMP. Patients should be instructed in the maintenance of good oral hygiene, using toothbrushes with soft or extra-soft bristles, applying the modified Bass brushing technique, and using dental floss. In their review, Garcia-Pola et al. also recommended rinsing with chlorhexidine twice daily, initially with a concentration of 0.2%, and a maintenance concentration of 0.12% for one to four weeks.

Information for patients
Written information is provided by the EADV webpage and the patient support groups. The purpose of these associations is to promote knowledge about the disease, to furnish comfort and share the experience of patients regarding daily life, and to disseminate information. Such information may contribute to a better overall management of the disease by promoting cooperation between patients, patient associations and health professionals. Patients are also informed about referral centres

Recommendations

It is recommended that patients and their families be informed about the disease, its clinical course and prognosis, treatment, relapse signs, and possible adverse events associated with treatment.
It is recommended that patients be informed about patient support groups for MMP (see list below).

List of support groups for patients with MMP:

International Pemphigus and Pemphigoid foundation: www.pemphigus.org

Pemphigus und Pemphigoid Selbsthilfegruppe e.V.: www.pemphigus-pemphigoid-selbsthilfe.de

Association Pemphigus Pemphigoïde-France: www.pemphigus.asso.fr

Pemfriends: www.pemfriends.co.uk

Associazione Nazionale Pemfigo/Pemfigoide:

Netwerk voor Blaarziekten: www.netwerkblaarziekten.nl

Pemfigus Hastaları Yardımlaşma ve Dayanışma Dernegi: www.pemfigus.org.tr

Future perspective and gaps in knowledge
Several important gaps in knowledge that exist were formulated by the guideline working group:
- Effectiveness and sequence of the different drugs used in MMP
- Ocular MMP: laser therapy and plugging eyelashes
- Validation of outcome measurements
- Scoring system for multisite MMP

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary material.