Mucous membrane pemphigoid with ocular involvement: the clinical phenotype and its relationship to direct immunofluorescence findings

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Running head

Autoantibody tests in mucous membrane pemphigoid diagnosis (52/60 characters)

Précis (35 words)

This study demonstrates that the clinical phenotype in direct immunofluorescence (DIF) positive and negative ocular MMP is very similar. This finding supports the rationale for the recognition of the diagnosis of DIF negative ocular MMP.

Abstract (348 of a 350 word limit)

Objective: This study explored the validity of the First International Consensus on Mucous Membrane Pemphigoid (MMP) guidance which recommends that clinically indistinguishable patients, who have direct immunofluorescence (DIF) negative biopsies, be excluded from a diagnosis of MMP. Misdiagnosis, or delayed diagnosis, of MMP with ocular involvement leads to the inappropriate use of topical therapy, the standard of care for causes of cicatrising conjunctivitis other than MMP, rather than systemic immunomodulatory therapy; resulting in irreversible clinical deterioration in MMP patients.

Design: Prospective cross-sectional study

Subjects and controls: 73 patients meeting the clinical criteria of ocular MMP, including those with positive and negative DIF findings.

Testing: A case report form was used to collect demographic details, the clinical history, and the results of a detailed clinical assessment by ophthalmologists, otolaryngologists, dermatology and oral medicine specialists. All anatomical sites, potentially affected by MMP, were examined apart from the oesophagus (and larynx in a subset). DIF results were recorded.

Main outcome measures: Differences between DIF positive and negative patients in demography, sites of involvement, and disease severity as determined by the degree of: conjunctival scarring (using Tauber staging), central corneal disease (vessels, scarring, ulceration and conjunctivalisation), history of conjunctival or lid surgery, and requirement for systemic immunotherapy at the time of screening.

Results: 73 patients with ocular MMP were recruited of whom 20/73 (27.4%) had ocular only disease. There was no significant demographic or clinical difference between patients with positive and negative DIF results. This finding included differences in disease severity for which the only significant difference was that of more severe central corneal disease in DIF negative patients. Asymptomatic disease at different sites was frequent.

Conclusions: These findings do not support the classification of DIF negative patients, meeting the clinical criteria for ocular MMP, as having a different disease. This category of patients should be accepted as having DIF negative MMP, for clinical management purposes, with patients having inflamed eyes being treated with systemic immunomodulatory therapy. The frequent finding of asymptomatic ocular, oral and nasopharyngeal MMP is clinically significant and implies that these sites should be routinely screened in asymptomatic patients.

INTRODUCTION

Mucous membrane pemphigoid (MMP), previously known as "cicatricial pemphigoid", refers to a heterogenous group of autoimmune subepidermal blistering disorders that affect mucous membranes at the orifices, including the ocular, oral, nasopharyngeal, tracheal, oesophageal, anogenital and genitourinary; the skin may or may not be affected.[1] Inflammation is associated with progressive cicatrisation (scarring) at all sites, with the exception of the oral mucosa, where scarring is uncommon. The reported incidence of MMP is approximately 1.16 to 2.0 per million population[2,3] and prevalence 1:40,000.[4] Approximately 70% of patients with MMP have ocular involvement (ocular MMP).[5,6] Ocular MMP, characterised by relapsing conjunctivitis with progressive conjunctival cicatrisation, is the commonest cause of cicatrising conjunctivitis in the United Kingdom with an incidence of 0.8 per million population.[7] Although the mean age of onset of ocular MMP is 65 years,[8,9] it also occurs in children and young adults in whom the disease is more aggressive.[10-12] The current standard of care for patients with symptomatic ocular MMP is systemic immunomodulatory therapy, because of the failure of topical therapies in MMP affecting this site.[11,13-15] However, the response to systemic immunomodulatory therapy is variable, and side effects are common.[8,16] Chronic discomfort is normal, and 20% of cases become bilaterally blind due to ocular surface failure, corneal vascularisation, and corneal opacification.[16,17]

Early diagnosis and treatment are essential to reduce sight-threatening complications in ocular MMP. It is recommended that a clinical diagnosis of MMP is made only when the clinical criteria for MMP at any site are accompanied by laboratory evidence of an antibody mediated disease at the epithelial basement membrane.[1] The latter requires a biopsy from any mucosal site (not necessarily ocular), or from skin, demonstrating linear deposition of IgG and/or IgA and/or complement at the epithelial basement membrane (BM) using a direct immunofluorescence (DIF) technique.[1,18-20]

Ocular MMP limited to the eye (ocular only MMP) has varied from 14/74 (19%) to 26/86 (30%) depending on the definition in one study[9] and 18/50 (36%) in another.[7] However, it is recognised that in ocular only MMP, half of the patients with conjunctival disease typical of MMP, have had intermittent or repeatedly negative DIF.[9,15,21-24] This may result in delayed or incorrect diagnosis. Because the standard of care for cicatrising conjunctivitis, other than that caused by MMP, is with topical as opposed to systemic immunomodulatory therapy, these patients can progress irreversibly. For these reasons we have previously proposed that a clinical diagnosis of ocular only MMP, in patients with a negative biopsy result, can be made in patients meeting the clinical criteria for MMP, after excluding other causes of conjunctival scarring.[8,25] This proposal has not been widely accepted as it is counter to the guidance in the First International Consensus on Mucous Membrane Pemphigoid,[1] which recommends that clinically indistinguishable patients, who have direct immunofluorescence (DIF) negative biopsies, be excluded from a diagnosis of MMP. Negative immunopathology findings have been thought to occur in this group of patients because antibody levels are low, and frequently undetectable, because the sensitivity of DIF in the conjunctiva is low for reasons that are uncertain[23] or because the disease is not MMP although alternative diagnoses have not been offered.[1] It is also

possible that BM autoantibodies may be absent in a subset of MMP patients who have developed disease due to an autoreactive T cell mediated immune response, without the development of detectable autoantibodies.[15]

This prospective cross sectional study was designed to explore the hypothesis that DIF negative ocular MMP might represent a different disease subset from those with a positive DIF results, by exploring differences in the phenotype of these patients. Parameters compared included the demography, distribution of sites of involvement, severity, and activity of the ocular disease. Patients with asymptomatic disease at different sites were also recorded.

METHODS

This was a prospective cross-sectional study on a cohort of patients diagnosed with MMP. The study protocol has been approved by the UK National Research Ethics Service (Reference 09/H0721/54). The study adhered to the tenets of the Declaration of Helsinki.

Patients diagnosed with MMP, at any site, were identified from databases of existing patients, and from new referrals, at two London Clinics (Moorfields Eye Hospital NHS Foundation Trust, Corneal and External Disease Clinics and Guys and St Thomas's NHS Foundation Trust, Oral Medicine and Dermatology Clinics). Patients had the following sites assessed for the presence of MMP: ocular, oral, skin, anogenital and nasopharyngeal by the relevant specialists. Nasopharyngeal screening was carried out in the otolaryngology departments at both Guys and St Thomas's NHS Foundation Trust and at the Royal National Ear Nose Throat Hospital. The results of previous DIF tests were recorded as positive or negative. If DIF had not been carried out previously, biopsies from affected mucosa or skin were taken and processed for DIF using standard techniques.[26] We were unable to standardize the DIF method because many patients had been referred with DIF results from biopsies that had performed locally. Details of the DIF findings were not available for all the patients and were not recorded. For this study, the diagnosis of ocular MMP was based on clinical findings typical of ocular MMP (after exclusion of other causes of scarring conjunctivitis),[8,15] regardless of DIF results.

Data collection used a case report form designed for this study (Supplementary Appendix 1, online). A clinical history was taken from all patients, focusing on their general health and the involvement of other anatomical sites by MMP. Other information obtained included demographic details, a medical history of autoimmune diseases or malignancy, and the ophthalmic history.

All patients then underwent a detailed clinical assessment by a multi-disciplinary team of ophthalmologists, otolaryngologists and a dermatology and oral medicine specialist. All anatomical sites that can potentially be affected by MMP, apart from the oesophagus (and larynx in a subset of patients), were screened for signs of disease. Fourteen patients declined nasopharyngeal and anogenital examination. When a patient declined screening of particular anatomical sites (apart from the eye), site involvement was determined from the disease history. History is necessary because for most oral disease cases, and some with nasopharyngeal involvement, there is no residual scarring to indicate a disease episode in patients in remission. Table 1a summarises the sites assessed for involvement by

MMP and the positive screening criteria for each site. Table 1b describes the classification used MMP involvement of sites using both screening and history.

Ophthalmological Assessment

During ophthalmological assessment, the best corrected visual acuity for each eye was recorded in Snellen's notation. A score was given to each eye according to its visual acuity: 1=6/7.5 or better, 2=6/9-6/12, 3=6/18-6/36, 4=6/60 or worse, 5=3/60-count fingers, 6=hand movements, 7=perception of light, and 8=no perception of light. For each patient, the score from the eye with the worst visual acuity was used for analysis.

Each eye was given an inflammation scoring methodology in the case report form (Supplementary Appendix 1 online). The score for each quadrant of bulbar conjunctiva ranged from 0-4 giving a maximum score of 16 for each eye, and of 32 for both eyes. A patient was defined as having significant ocular inflammation if the total score was 5 or more: minimal levels of conjunctival inflammation may be due to blepharoconjunctivitis or dry eye rather than to underlying MMP related inflammation.

Tauber staging was used to assess the extent of conjunctival scarring.[27] All patients had conjunctival scarring by definition. Severe scarring was defined as Tauber stage greater than IIb (lower fornix shortening more than 25%) or Tauber stage greater than IIIb (presence of lower lid symblepharon more than 25%).

Amongst the other indices of severity assessed were corneal pathologies expected to reduce vision: vascularisation, scarring, ulceration, and conjunctivalisation. Severe disease was classified as any of these involving the central 5mm of cornea (pupillary zone).

Ocular discomfort as reported by patients were graded as: none, tolerable, moderate, or severe. The extent to which vision affects daily activities as reported by patients were graded as: unaffected, adequate for needs, and restricts activity.

Statistical analysis

Data were managed in Excel (Microsoft) and analysed using Statistical Program for Social Sciences (SPSS©) Version 22 (2013 IBM© US). Differences in the distribution of categorical variables between groups were analysed using the Chi-squared test. Fisher's exact test was used when expected frequencies of cells less than 5 were present. For continuous variables, differences in distributions between DIF positive and DIF negative groups were analysed using the Mann-Whitney U test. Significance level was set at $p < 0.05$.

RESULTS

112 patients with a diagnosis of MMP were recruited. 73/112 (65.2%) patients screened had ocular involvement and it is these that have been evaluated for this study. The median time from the diagnosis of MMP to the study examination was 104 months (interquartile range [IQR] 54 – 146 months). The data for each patient are included in Supplementary Table 1 online.

Patient characteristics and direct immunofluorescence findings

The presenting features of the 73 patients with ocular MMP are summarised in Table 2. There were no significant demographic or clinical differences between DIF positive and DIF negative patients.

Differences between patients grouped by differences in involvement at different sites

Table 3 describes the sites involved in this group of ocular MMP patients and compares their demographic characteristics, the numbers using systemic immunotherapy at the time of screening, those who had asymptomatic disease identified at screening, and the DIF results. Asymptomatic disease at different sites was common and identified in 8/19 (42.1%) patients with ocular and oral disease and 6/10 (60.0%) patients with ocular, oral and nasopharyngeal disease. *Of those with evidence of mucosal involvement in the nasopharynx, 6/10 (60%) were asymptomatic*. Compared to patients in other groups, patients who had ocular only involvement were more likely to have a negative DIF status (p=0.03). Figure 1 describes the patients grouped by different sites of involvement: 20/73 (27.4%) had ocular only disease, 19/73 (26.0%) had ocular and oral disease, 10/73 (13.7%) had ocular, oral and nasopharyngeal disease, and 24/73 (32.9%) had ocular disease with multiple extraocular sites involved in various other combinations. The anatomical sites involved were classified using the criteria described in Table 1.

Severity of disease comparison in DIF positive versus DIF negative ocular MMP patients

Table 4 and Figure 2 compare the severity of disease in having positive and negative DIF results. For all cases of ocular disease there was a trend to more severe disease in DIF negative patients, with differences that were statistically significant for the presence of central corneal disease.

For the 19 patients with ocular only MMP, disease severity indices (Table 4) were evenly balanced with trends to less conjunctival scarring in the DIF negative group but worse corneal disease, and a very similar requirement for systemic immunotherapy.

Visual acuity scores were statistically significantly worse in DIF negative patients (p=0.03). However, due to clinically significant ocular co-morbidities in these patients, visual acuity scores can be difficult to interpret. There was no statistically significant difference in the proportion of patients who reported restriction of daily activities due to poor vision (p=0.258). Reported ocular discomfort scores were similar in both DIF positive and DIF negative patients (p=0.104).

DISCUSSION

This study of patients having ocular MMP were also assessed for the presence of MMP at extraocular sites. Limitations of this study are the inclusion of patients who declined screening and examination of particular anatomical sites, such as nasopharyngeal and anogenital regions, for whom the assumption was made that these sites were uninvolved in the absence of a history for MMP at that site. Although the clinical signs and scarring parameters in the case report form used in this study were based on previously published systems,[27] these are not validated. There is currently no validated scheme for measuring the severity and activity of disease in scarring conjunctivitis. In addition, this was a cross

sectional study so that, although we could record the requirement for systemic immunosuppressive therapy at the time of the study, we could not assess the effect of treatment on outcomes. However, in our previous study the datasheet evaluating long term outcomes is included in the supplementary data.[16] We have used this to assess the effect of the first treatment episode in DIF positive and negative ocular MMP patients respectively: these data are shown in Supplementary Figure 1 which show no major differences in the outcomes. Immunosuppressive treatments were administered according to previously published step-wise regimen.[15]

We have shown that a substantial proportion of cases had ocular only disease (20/73 [27.39%]) without involvement of other sites: similar to that in previous reports.[7,9,17] We also confirmed that in this subset with ocular only disease, 11/19 (55%) were significantly less likely (p=0.03) to have a positive DIF result. This proportion is similar to what has been previously described for this group of patients.[9,15,21-24] Twenty six patients (35.6%) of the overall ocular MMP cohort were DIF negative. This is the first prospective study to provide a detailed analysis of clinical differences between patients, meeting clinical criteria of ocular MMP, irrespective of their DIF findings. This has shown that there are few differences between the DIF negative and DIF positive subsets, with two parameters, central corneal pathology and prior lid or conjunctival surgery, being significantly more common in the DIF negative group. This evidence provides no support for the classification of DIF negative MMP as a different disease from DIF positive MMP as has been suggested[1] and is in keeping with findings in two other studies exploring the issue of DIF negative ocular MMP patients[23,24].

This is also the first study, to our knowledge, that has reported the results of screening of asymptomatic sites in MMP patients. Asymptomatic ocular, oral and nasopharyngeal disease were frequently identified. This finding is clinically significant as some ocular MMP patients develop progressive cicatrisation without clinical inflammation,[28] discomfort from MMP may be accepted as a matter of course by patients having oral MMP who can be expected to benefit from appropriate management. Lastly, asymptomatic tracheal involvement has been reported in a series of patients with nasopharyngeal disease[29] and can lead to severe complications which may benefit from early identification.

Our demonstration that, in patients meeting clinical criteria for ocular MMP the direct immunofluorescence findings do not relate to the clinical phenotype, supports our previous recommendation that clinical criteria, together with the result of conjunctival biopsies for both routine histopathology and DIF, can be used to make a definitive diagnosis of DIF negative ocular MMP. This is justified because the more than 20 other diseases causing conjunctival scarring can be excluded with a combination of these biopsy results and clinical criteria. Histopathology is needed to exclude ocular surface tumors whereas DIF is used not only to confirm a diagnosis of MMP, but also to distinguish MMP from other causes of conjunctival scarring; these include lichen planus, showing shaggy discontinuous fibrinogen deposits at the BMZ,[30-32] and pemphigus having intraepithelial antibodies.[1] The infrequent cases of inflammation and progressive scarring, associated with both Stevens Johnson syndrome and topical medications (following withdrawal of the medication) that are DIF negative should also be treated in the same way as DIF negative MMP. If DIF is negative then alternative evidence of autoantibodies to epithelial BM proteins may be available from autoantibody detection,

using indirect immunofluorescence, ELISA or Western blotting. However these tests are often negative, and therefore not required for diagnosis using the Consensus criteria;[1] in our experience they are also usually negative in DIF negative patients. Diagnostic criteria for the many causes of cicatrising conjunctivitis, and a flow chart for this, have been described in detail in a recent review.[15]

Whereas we agree that in ocular only DIF a conjunctival biopsy should be taken for DIF testing, and that for some purposes, such as the investigation of some aspects of the immunopathogenesis of conjunctival MMP, tissue should only be used from patients having at least one positive DIF result[33-35], we hope that the diagnosis of DIF negative ocular MMP will be widely accepted for clinical management purposes. This will allow patients with this condition to access appropriate therapy without the delays that are currently common, because of failure to meet the existing diagnostic criteria for MMP affecting other sites.

Moreover, performing immunofluorescence on small conjunctival samples can be operator-dependent and the interpretation of immunofluorescence results subjective. Thus, technical and interpretation factors may contribute to both false negative and false positive DIF findings. Furthermore, the absence of identifiable autoantibodies in some patients with clinical MMP may not only be due to undetectably low levels of antibody but also suggests the possibility that a subset of MMP patients have disease that results from a cell mediated response resulting from autoreactive T cells to epithelial basement membrane proteins, without circulating antibodies. This would parallel the situation in most other autoimmune diseases which result from variable levels of cellular and autoantibody driven responses.[15] We think that this hypothesis deserves further investigation in MMP.

FIGURE LEGENDS

Figure 1:

Cohort of patients with ocular mucous membrane pemphigoid showing the combinations of sites (n=73).

Figure 2:

Direct immunofluroescence (DIF) status and severity of disease showing trends of more severe disease in 26 DIF negative patients compared to 43 DIF positive patients.

Supplementary Figure 1:

Bar graph showing first treatment episode outcomes for biopsy-positive and biopsy-negative patients. x-axis: percentage success, qualified success, failure in patients given that agent; y-axis: principal agent. DIF = direct immunofluorescence; n = no. of patients; *p-values compares distribution of drug therapies between DIF positive and DIF negative patients, Fisher's exact (2-sided) test. There were no significant differences in treatment outcomes between DIF positive and DIF negative patients for all drug categories combined (p=0.702).

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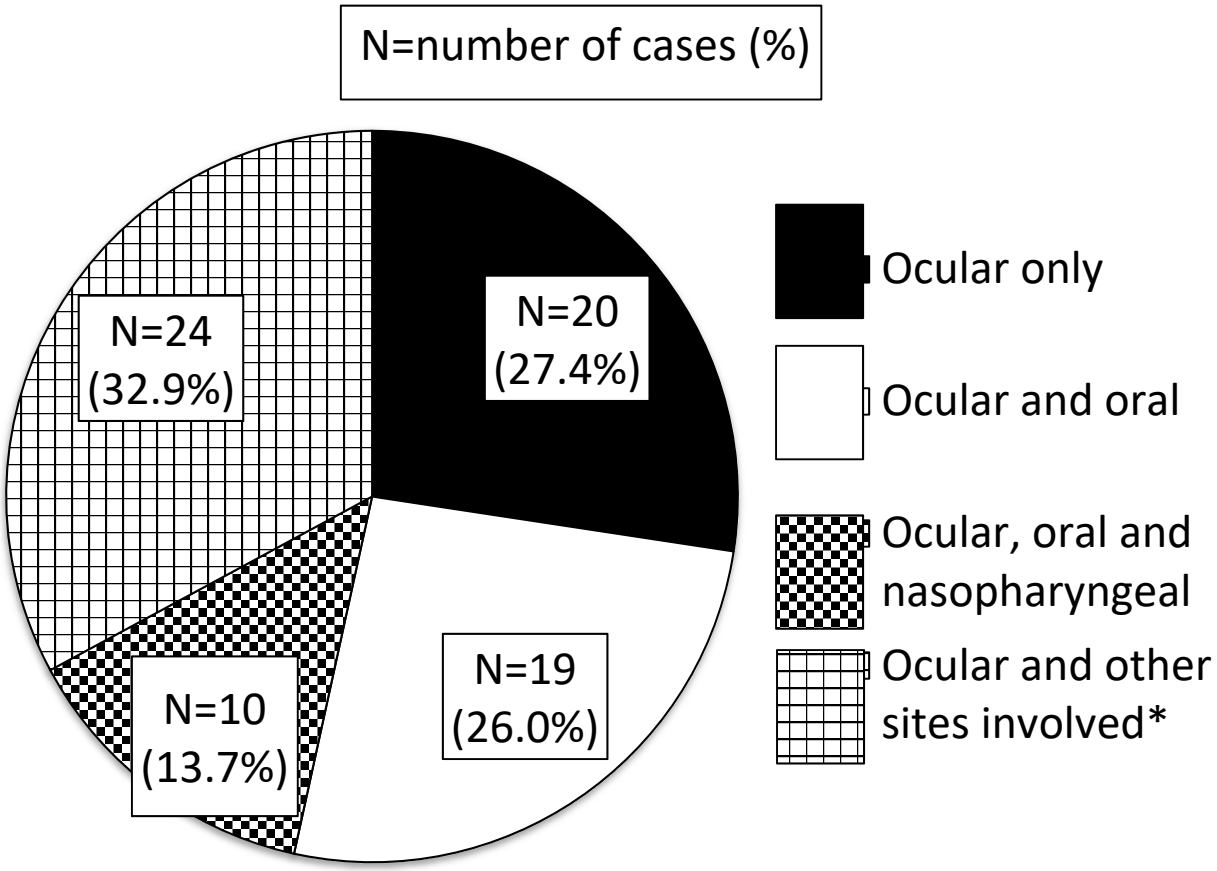
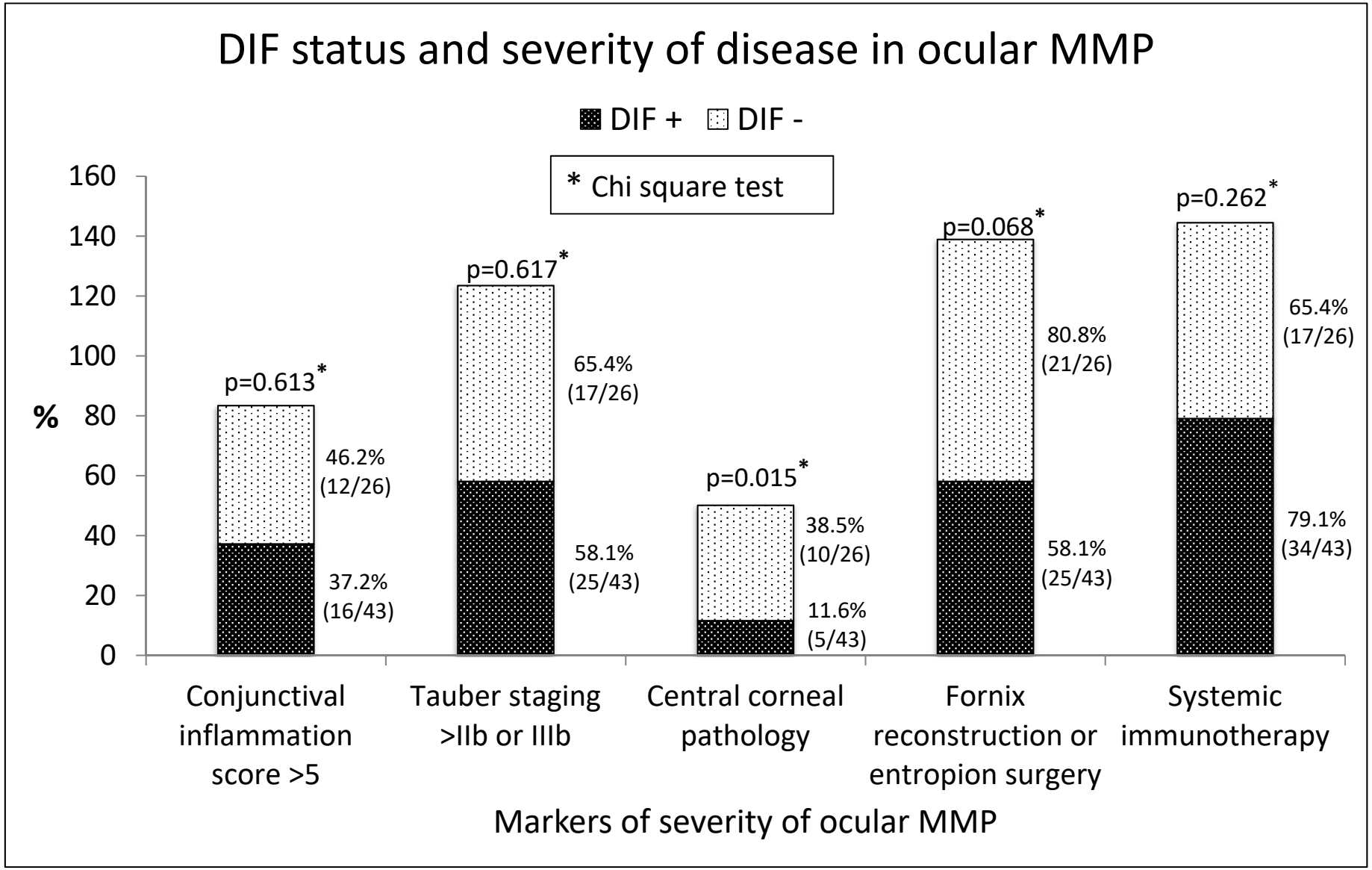
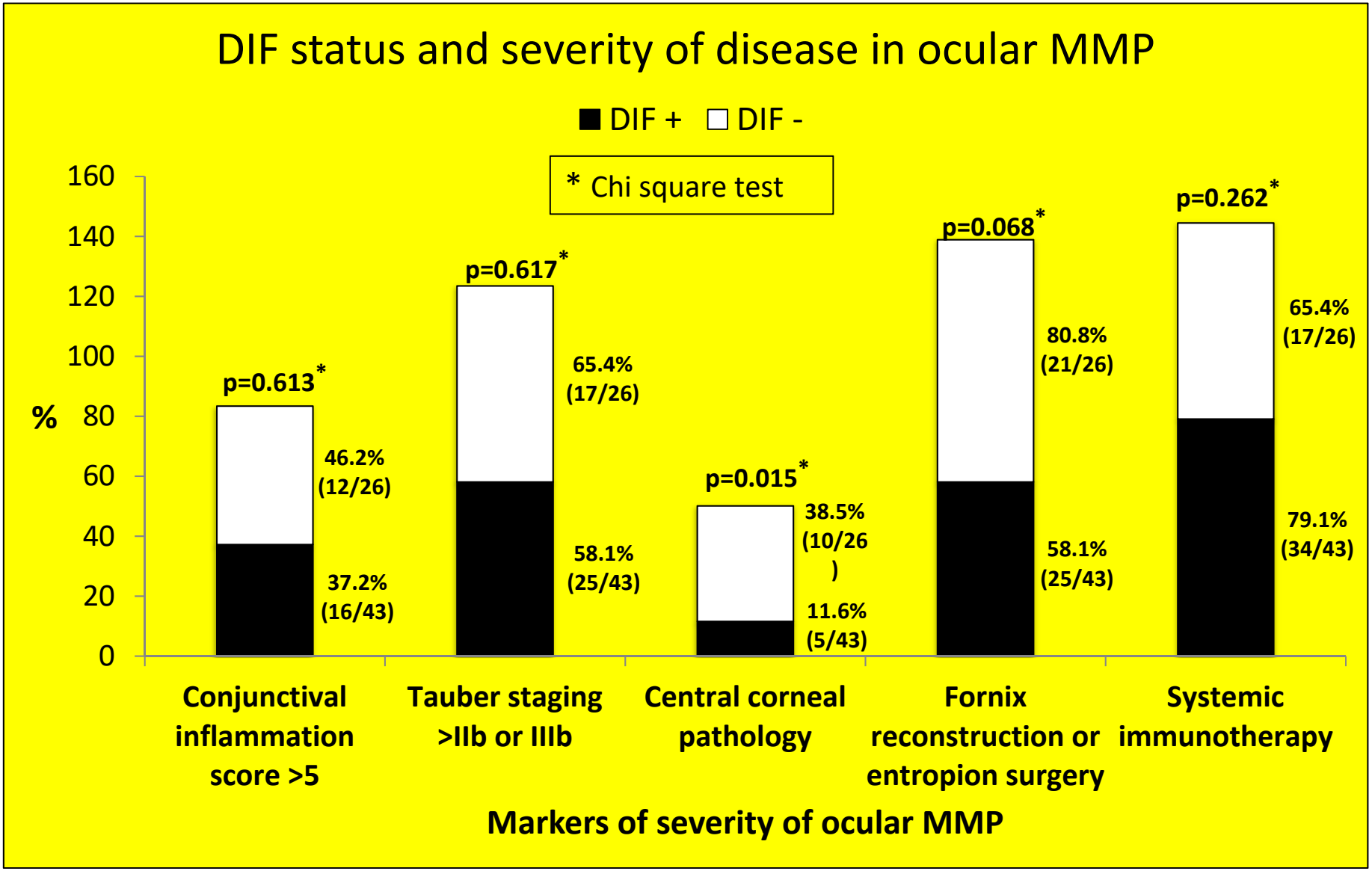
Sites involved in mucous membrane pemphigoid with ocular involvement



**Ocular and other sites involved* (Detailed breakdown of sites)	*Number of patients (%)* 24 (32.9%)
Ocular, oral & skin	6
Ocular, oral nasopharyngeal & skin	6
Ocular, oral & anogenital	3
Ocular, oral, nasopharyngeal & anogenital	2
Ocular, oral, nasopharyngeal, anogenital & skin	2
Ocular and skin	2
Ocular, oral, anogenital & skin	1
Ocular & nasopharyngeal	1
Ocular & anogenital	1


DIF status and severity of disease in ocular MMP
DIF +
DIF -
* Chi square test
160
140
120
100
%
80
60
40
20
0
p=0.613*
46.2% (12/26)
37.2% (16/43)
p=0.617*
65.4% (17/26)
58.1% (25/43)
p=0.015*
38.5% (10/26)
11.6% (5/43)
p=0.068*
80.8% (21/26)
58.1% (25/43)
p=0.262*
65.4% (17/26)
79.1% (34/43)
Conjunctival inflammation score >5
Tauber staging >IIb or IIIb
Central corneal pathology
Fornix reconstruction or entropion surgery
Systemic immunotherapy
Markers of severity of ocular MMP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Systemic immunotherapy
Markers of severity of ocular MMP

Table 1a Sites assessed for involvement by mucous membrane pemphigoid (MMP) and positive screening criteria for MMP involvement of sites

Site	Sites assessed	Positive screening criteria for MMP at each site
Ocular	Lids, conjunctiva and cornea	Conjunctival scarring mandatory for diagnosis
Oral	Lips, buccal mucosa, gingivae, tongue, floor of mouth, hard palate, oropharynx	Erythema, ulceration or scarring AND/OR a history of oral MMP (included as signs of inactive disease are usually absent because residual scarring is uncommon)
Nasopharyngeal	Nasal cavity, nasopharynx, oropharynx, hypopharynx and larynx	Crusting and/or ulceration and/or scarring at each site
Genital	Labia major/minor, vestibule, vagina, glans penis, prepuce	Erosions and/or scarring at any site
Skin	Skin	Ulcers and/or scars

Table 1b Classification for mucous membrane pemphigoid involvement of sites by screening and history*

Ocular only	**Definitive clinical**	Screen positive, & no History of non-ocular MMP, & ALL other sites screened and found free of MMP
	Probable clinical	Screen positive, & no History of non-ocular MMP, & no MMP in other sites screened but 1 or more other sites NOT screened.
Oral only	**Definitive clinical**	Screen positive &/or History of oral MMP, & no History of other site involvement, & ALL other sites Screened and found free of MMP.
	Probable clinical	Screen positive &/or History of oral MMP, & no History of other site involvement, & no MMP in other sites but 1 or more other sites NOT screened.
Ocular & Oral only	**Definitive clinical**	Screen positive ocular & [Screen positive oral **OR** History of oral], & no History of other site involvement, & ALL other sites Screened and found free of MMP.
	Probable clinical	Screen positive ocular & [Screen positive oral **OR** History of oral], & no History of other site involvement, & no MMP in other sites but 1 or more other sites NOT Screened.
Nasopharyngeal ± other sites **Genital ± other sites** **Skin ± other sites**	**Definitive clinical**	Screen positive or History positive or both
	Absent	Screen negative & History negative
	DS/Uncertain	Declined screening (DS) / not screened for the site

***** The presence of scarring was mandatory for a diagnosis of ocular MMP. At the other sites disease may resolve without scarring (particularly in the oral mucosa): a history of disease at the extraocular sites was therefore a criterion for a definitive clinical diagnosis

Table 2 Patient characteristics and direct immunofluorescence status.

Baseline characteristics	DIF* positive (n=43)	DIF* negative (n=26)	DIF* unknown/uncertain (n=4)	Significance‡
Age of diagnosis in years (range [R], median [M], interquartile range [IQR])	[R] 18 - 86, [M] 58, [IQR] 52 - 64	[R] 23 - 82, [M] 60.5, [IQR] 51 - 71	[R] 53 - 70, [M] 66.5, [IQR] 59.5 - 68.5	p = 0.620§
Females	14 (32.6%)	12 (46.2%)	1 (25.0%)	p = 0.259 ‖
Race				
White-British	33 (76.7%)	21 (80.8%)	4 (100.0%)	
White-Irish	2 (4.7%)	0 (0.0%)	0 (0.0%)	
White-Other	2 (4.7%)	1 (3.9%)	0 (0.0%)	
Black-African	0 (0.0%)	1 (3.9%)	0 (0.0%)	p = 0.566**
Asian-Indian	1 (2.3%)	1 (3.9%)	0 (0.0%)	
Asian-Pakistani	1 (2.3%)	0 (0.0%)	0 (0.0%)	
Other	1 (2.3%)	2 (7.7%)	0 (0.0%)	
Unknown	3 (7.0%)	0 (0.0%)	0 (0.0%)	
Time from diagnosis in months†† (range [R], median [M], interquartile range [IQR])	[R] 26 - 325, [M] 87.0, [IQR] 54 – 141	[R] 19 - 345,[M] 123.5, [IQR] 55.5 - 176.5	[R] 22 - 173, [M] 87.5, [IQR] 25.5 - 164.5	p = 0.373§
Autoimmune disease‡‡				
Yes	16 (37.2%)	8 (30.8%)	3 (75.0%)	p = 0.586 ‖
No	27 (62.8%)	18 (69.2%)	1 (25.0%)	
Malignancy				
Yes	6 (14.0%)	3 (11.5%)	0 (0.0%)	p >0.999**
No	37 (86.1%)	23 (88.5%)	4 (100.0%)	
Ocular co-morbidities				
Glaucoma	8 (18.6%)	4 (15.4%)	1 (25.0%)	> 0.999**
Pseudophakia	13 (30.2%)	14 (53.8%)	0 (0.0%)	0.075**
Previous lid surgery	21 (48.8%)	13 (50.0%)	1 (25.0%)	> 0.999**
Previous conjunctival surgery	6 (14.0%)	6 (23.1%)	0 (0.0%)	0.347**
Previous glaucoma surgery	1 (2.3%)	0 (0.0%)	1 (25.0%)	> 0.999**
Corneal graft	0 (0.0%)	3 (11.5%)	0 (0.0%)	0.0496**
Other eye surgery	4 (9.3%)	4 (15.4%)	0 (0.0%)	0.464**
Other eye disease	1 (2.3%)	1 (3.9%)	0 (0.0%)	> 0.999**

*Direct immunofluorescence results †Oral, nasopharyngeal, skin, anogenital involvement in various combinations ‡Comparing DIF postive and DIF negative
§Mann-Whitney U test ‖Chi-square test **Fisher's exact test (2-sided) ††Time of follow-up from diagnosis ‡‡Includes thyroid disease, type 1 diabetes mellitus, rheumatoid arthritis, psoriasis, lichen planus, Sjogren's syndrome, systemic lupus erytheomatosuus, atopy, and other autoimmune diseases.

Table 3 Sites involved[*] and patient characteristics of ocular mucous membrane pemphigoid (OcMMP) cases and direct immunofluorescence (DIF) results.

Characteristics	All ocular MMP	Ocular only MMP	Ocular & oral MMP	Ocular, oral, and nasopharyngeal MMP	Ocular + other combinations of extraocular sites involved[†]
Total (n)	73	20	19	10	24
Female	27 (37.0%)	9 (45.0%)	5 (26.3%)	6 (60.0%)	7 (29.2%)
Age (years)					
Median	60	67.5	58.0	55.0	60.0
Interquartile range	53 - 68	52 - 77.5	51.0 - 62.0	40.8 - 61.2	55.3 - 68.0
White race	63 (86.3%)	18 (90.0%)	17 (89.5%)	10 (100.0%)	18 (75.0%)
Systemic immunotherapy	52 (71.2%)	15 (75.0%)	12 (63.2%)	10 (100.0%)	15 (62.5%)
Asymptomatic of site(s) involved	-	0 (0.0%)	Ocular 5 (26.3%)[‡] Oral 4 (21.1%)[§]	Ocular 0 (0.0%) Oral 2 (20.0%)[‖] Nasopharyngeal 6 (60.0%)[**]	-
DIF Results: Significantly fewer patients with ocular only MMP were DIF positive (p=0.03)[††]					
DIF +	43 (58.9%)	8 (40.0%)	15 (79.0%)	6 (60.0%)	14 (58.3%)
DIF -	26 (35.6%)	11 (55.0%)	4 (21.1%)	4 (40.0%)	7 (29.2%)
DIF unknown	4 (5.5%)	1 (5.0%)	0 (0.0%)	0 (0.0%)	3 (12.5%)

DIF + = Direct immunofluorescene positive; DIF - = Direct immunofluorescence negative; [*]Sites involved detected at the time of this cross-sectional study; some sites may be in remission; [†]Oral, nasopharyngeal, skin, anogenital involvement in various combinations; [‡]5 were DIF +; [§]3 were DIF + and 1 was DIF -; [‖]2 were DIF -; [**]4 were DIF + and 2 were DIF -; [††]Chi-square test.

Table 4 Indices of disease activity and severity for ocular and relationship to direct Immunofluorescence (DIF) findings.

Direct immuno-fluorescence (DIF) result	**OCULAR indices of disease activity and severity (any case of ocular disease +/- other sites involved)**				
	Index of ocular disease activity	**Indices of severity of disease**			
	Ocular inflammation score ≥ 5[*] (n, %)	**Tauber stage >IIb, IIIb**[†] (n, %)	**Central corneal conditions**[‡] (n, %)	**History of fornix reconstruction or entropion surgery** (n, %)	**Systemic immunotherapy ocular patients** (n, %)
Positive	16/43 (37.2%)	25/43 (58.1%)	5/43 (11.6%)	25/43 (58.1%)	34/43 (79.1%)
Negative	12/26 (46.2%)	17/26 (65.4%)	10/26 (38.5%)	21/26 (80.8%)	17/26 (65.4%)
p-values[‖]	*0.613*	*0.617*	***0.015***	*0.068*	*0.262*
	OCULAR indices of disease activity and severity (ocular only disease with no other sites involved)				
	Index of ocular disease activity	**Indices of severity of disease**			
	Ocular inflammation score ≥ 5[*] (n, %)	**Tauber stage >IIb, IIIb**[†] (n, %)	**Central corneal conditions**[‡] (n, %)	**History of fornix reconstruction or entropion surgery** (n, %)	**Systemic immunotherapy ocular patients** (n, %)
Positive	5/8 (62.5%)	6/8 (75.0%)	3/8(37.5%)	8/8 (100.0%)	7/8 (87.5%)
Negative	4/11 (36.4%)	7/11 (63.6%)	5/11 (45.5%)	9/11 (81.8%)	9/11 (81.8%)
p-values[‖]	*0.370*	*>0.999*	*>0.999*	*0.485*	*>0.999*

[*] Inflammation score using the Moorfields & Institute of Ophthalmology conjunctival inflammation grading system for ocular mucous membrane pemphigoid; score for each bulbar conjunctival quadrant 0=None, 0.5-1.0=Minimal, 1.5-2.0=Mild, 3.0-3.5=Moderate, 4.0=Severe (maximum 16 for each eye); [†]Tauber staging >IIb=lower fornix foreshortening >25%, >IIIb=presence of lower lid symblepharon>25%; [‡] Central corneal conditions include central vessels, central scarring, central ulceration, central conjunctivalisation; [‖]Fisher's exact test (2-sided).