Autoantibody detection for diagnosis in direct immunofluorescence negative mucous membrane pemphigoid: ocular and other sites compared.

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Running head (54/60 characters)
Serum autoantibody tests in the diagnosis of ocular pemphigoid.

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Short abstract

Objective: To assess whether a panel of serum pemphigoid autoantibody tests could be used to confirm an immunopathological diagnosis of mucous membrane pemphigoid (MMP) in direct immunofluorescent negative (DIF-) MMP patients.

Design: Prospective cross-sectional study.

Subjects and controls: 76 patients with MMP involving ocular and non-ocular sites with 45 matched controls.

Tests: Enzyme linked immunosorbent assays (ELISA) for BP180 and BP230 (MBL International®), IgA and IgG indirect immunofluorescence on human salt-split skin (IIF SSS) and the keratinocyte footprint assay for anti-laminin 332 antibodies.

Main outcome measures: Sensitivity and specificity of autoantibody detection; significant differences for individual tests and test combinations for MMP involving different sites.

Results: All DIF- cases (24/76, 31.8%) had either ocular only disease or ocular involvement in multi-site disease. Serum pemphigoid autoantibodies were detected in 29/76 (38.2%) of all MMP patients compared to 3/45 (6.7%) of controls. Autoantibody reactivity detected by any one or more of the tests was present in 6/24 (25%) DIF- cases compared to 22/49 (44.9%) in DIF positive (DIF+). Compared to controls ocular only MMP serum reactivity was not significantly different for any test or test combination whereas DIF- multisite ocular MMP differed for one ELISA and 3/7 test combinations. By contrast, for DIF+ non ocular MMP all the individual tests, apart from IgA IIF, and all test combinations were significantly different compared to controls. For the whole MMP cohort the sensitivity of all tests was low having a maximum of 21.05% for BP180 reactivity, increasing to 38.16% for an optimal test combination. Disease activity was strongly associated with positive serology findings.

Conclusions: Pemphigoid serum autoantibody tests did not provide alternative immunopathological evidence of MMP in ocular only MMP patients but had limited value in DIF- multisite ocular MMP. The requirement for immunopathological confirmation of MMP by autoantibody detection is inappropriate for DIF- ocular only MMP resulting in missed diagnoses, delayed therapy and poor outcomes. Alternative diagnostic criteria for MMP with ocular involvement are required, to exclude the other causes of scarring conjunctivitis, until more sensitive and specific immunopathology tests become available.

Introduction

Mucous membrane pemphigoid (MMP) is an autoimmune subepidermal blistering disease. Autoantibodies are usually present and directed against different components of the epithelial basement membrane (BM) of the mucosal orifices, with or without skin involvement. All pemphigoid disorders with predominant involvement of mucous membranes are termed MMP.[1] MMP patients with lesions limited to ocular and oral sites site have been termed ocular only MMP, synonymous with pure ocular MMP[2], or oral only MMP.[3] The conjunctiva is involved in two thirds of MMP cases.[1]

MMP diagnosis currently requires both clinical criteria and a biopsy showing IgG, IgA or complement at the epithelial basement membrane zone (BMZ), indicating the presence of autoantibodies, using either direct immunofluorescence (DIF) or immuno-histochemistry/immuno-electron microscopy. A positive DIF (DIF+) finding from any one site is accepted as diagnostic immunopathology evidence for disease at any other site that meets the clinical criteria for MMP.[1] Biopsies for DIF are taken from perilesional tissue of affected sites[1] including, where possible, uninflamed conjunctiva but biopsies may also be positive from clinically unaffected sites.[4] However, biopsies cannot always be taken for DIF (refused consent or inaccessible conjunctiva in advanced ocular disease) and, furthermore, are less sensitive in ocular only MMP than for MMP at other sites[5-7] with positive results in only around 50% of patients despite the use of multiple biopsies.[3,8] When the DIF result is negative (DIF-) or unavailable, the detection of circulating epithelial basement membrane autoantibodies in serum can be used to confirm the diagnosis.[1] In MMP, six target antigens been recognised as pemphigoid autoantibodies including BP180 (also termed collagen type XVII), BP230, laminin 332 for which tests are widely available.[9-14] Pemphigoid autoantibodies have been detectable in variable proportions of MMP patients from as low as 10% for IIF SSS, and zero for immunoblotting or immunoprecipitation for BP180 and BP230, in a subset of 10 ocular only MMP[15] to as high as 84%[16] of MMP patients having mixed site involvement.

Our primary hypothesis was that a panel of serum pemphigoid autoantibody tests, and their combinations, might be used to confirm an immunopathological diagnosis of MMP in DIF negative patients with ocular involvement. The hypothesis was tested by evaluating the sensitivity and specificity of tests and their combinations for cases with that of age, sex, race matched controls.

Methods

The study was approved by the UK Research Ethics Service Ref 09/H0721/54 and adhered to the tenets of the Declaration of Helsinki. It was a prospective cross-sectional study of patients diagnosed with MMP, and an age, sex, race matched control population, who donated blood for these serological studies. Patients and controls were recruited between 21/12/2009 and 05/08/2011.

Cases. MMP patients were recruited from both existing patients, and from new referrals, at two London clinics (Moorfields Eye Hospital NHS Foundation Trust, Corneal and External Disease Clinic and Guys and St Thomas's NHS Foundation Trust, Oral Medicine and Dermatology clinics). The results of previous DIF tests were recorded and, if these had not been carried out, a biopsy was taken and processed for DIF using standard techniques[17]. The diagnosis of MMP for cases with ocular involvement, without a positive DIF result, was based on the clinical and pathology criteria that we have previously proposed for this subset of patients.[3,8,18] Data were collected using a case report form designed for this study.[3] All MMP patients had a history taken, focusing on previous involvement of sites by MMP and their general health, and had an examination for signs of MMP at all potential anatomical sites, apart from the esophagus, by ophthalmologists, a dermatologist and oral medicine specialist, and otolaryngologists. Some patients declined the additional examinations for screening of extraocular sites (13 oral, 14 skin, 37 nasopharyngeal, 15 genital and 16 perianal). The history of disease at all sites was used to classify patients by site of involvement, both those whose disease was in remission with no residual clinical signs (common in oral MMP), and when the additional examinations had been declined. The sites assessed for involvement by MMP, and screening criteria for involvement at these sites, have been described and tabulated.[3]

Controls: The number of controls in this study (45) was chosen *a priori* to give an 80% power to detect a difference in the proportions of BP180-NC16a autoantibodies. This was calculated using Wieland's data on age, sex stratified controls having detectable levels in 14/337 (4.15%)[19], and our pilot data in our MMP cases in which 8/32 (25%) had detectable levels. Age sex and race matched controls were recruited from healthy staff and patients who were having surgery for ocular conditions, without associated systemic disease.

Serology tests

The serology test results analysed in this study were duplicated in a Service laboratory in 2014/15 and in the laboratory of the Centre for Blistering Diseases, The University of Groningen in 2018/19; discrepancies between the two were retested at the St John's Institute of Dermatology Laboratory in 2019. This was done to resolve the issue of unreliable data provided by the Service laboratory that became apparent in 2018. The sera were stored at the UCL Institute of Ophthalmology, London at -80C until March 2018 and at -20C thereafter. Laboratory staff were masked to the clinical findings.

Table 1 describes the 5 tests carried out on the sera for all 76 cases and 45 controls. The 51 discrepancies between the Groningen and Service laboratory results were retested by St Johns. For the 45 cases for which the Groningen results were confirmed by St John's the Groningen results were used for the analysis. The Service laboratory findings were used for the remaining 6 tests after the discrepancies with Groningen were confirmed by two repeat tests at St John's. Laminin 332 reactivity was reported only for the Groningen keratinocyte footprint assay (KFA)[20] results. Indirect

immunofluorescence was carried out using human salt split skin although protocols varied as there is no standard for this test.[21] The MBL ELISAs were carried out according to the manufacturer's protocol but procedures differed between these laboratories with regard to the reporting of the results; at Groningen sera with ELISA's of ≥6 U/ml were retested up to twice more and the results scored as positive if at least 2 tests met the manufacturer's recommended cut off of ≥9 U/ml and negative if any two tests showed lower concentrations than this. At the Service laboratory subjects with a result of ≥9 U/ml were recorded as positive unless a test was only weakly positive when it was repeated and reported as positive when the repetition was positive, or negative if the repeat was negative. At St John's tests were reported as positive when the result met the manufacturer's recommended cut off of ≥9 U/ml.

Statistics: The Sensitivity (Sn) and Specificity (Sp) were computed for those autoantibody tests showing a significantly higher frequency of positive reactions in MMP compared to controls. Youden's Index (Sn% + Sp% - 100) was used to identify the 'best' diagnostic test, giving equal weights to Sp and Sn, and taking the clinical diagnosis of MMP as the 'Reference Standard'. Youden's index of 100% indicates a perfect diagnostic test and above 80% is an acceptable value for a "good" test. The above procedures were repeated for some combinations of different tests, whereby the serology result was regarded as positive when one or more tests of the combination gave a positive reaction. The aim was to explore combinations that improved Sn or Sp or gave a higher Youden's Index. The frequency of positive reactions in controls and in both DIF+ and DIF- MMP cases were also compared using the Fisher's exact test. The frequency of positive reactions in controls and MMP clinical phenotypes (MMP involving different combinations of sites) were compared using Fisher's exact test, as appropriate.

Results

Characteristics of MMP patients and controls, direct immunofluorescence and serum autoantibody results

Table 1 describes the serology tests and the results of both individual tests and test combinations for all cases combined compared to controls. Supplementary Table 1 provides full clinical and serology data for the individual patients and controls. This dataset is also available as an Excel Workbook at Mendeley Data https://data.mendeley.com/datasets/7pxbkx84r3/draft?a=02efd7af-8c11-4dc0-8be0-c45b93682bad including "Patient and Control dataset" in sheet 1 and serology results from all three laboratories in sheet 2. Table 2 summarises the demographic data and overall serology test positivity for subjects with different sites involved by MMP and by their DIF status. MMP cases and controls were similar in terms of age, sex and race distribution.

Direct immunofluorescence:

A DIF result was available in 73/76 MMP patients. Direct immunofluorescence was positive for at least one site in 49/73 (67.1%) of cases. We included the 24 patients with negative DIF, and the 3 for whom these results were not available, but who met our clinical criteria for a diagnosis of MMP[3]. All 24 DIF- patients had ocular involvement (Table 2).

Serum pemphigoid autoantibody tests: when all tests were evaluated for cases and controls at least one positive test was reported for 29/76 (38.2%) MMP cases: 22/49 (44.9%) direct IF positive cases tested positive versus 6/24 (25%) DIF- cases (Table 2).

Proportions of MMP cases and controls with positive serum pemphigoid autoantibodies for individual tests and test combinations.

For individual tests results for the whole patient group (see Table 1) only ELISA BP180-NC16a MBL (ELISA BP180 MBL) and IgG IIF SSS were significantly different from controls. Control sera were positive in two tests: 2/45 (4.44%) for the ELISA BP180 MBL and 1/45 for ELISA BP230 MBL. These findings are shown graphically in Figure 1. Test combinations (any one or more tests positive) had substantially higher sensitivities than any individual test, with similar specificities although sensitivities were still low (17.1-38.2%) contributing to a low Youden's index. ELISA BP180, combined with IIF on SSS for IgG and IgA and the Lam 332 assay was an optimal combination with a sensitivity of 36.8 and specificity of 95.56. When all 5 tests were combined the sensitivity rises slightly to 38.16 but with a slightly reduced specificity of 93.33 because of one control was positive for BP230.

Supplementary Expanded Table 2 online is expanded from Table 1 to include the serology test results for the following additional patient subsets compared to controls: DIF + and DIF- cases; the sites most frequently involved by MMP (ocular only, oral only, ocular and oral only, and all non-ocular sites); and results for DIF+ non-ocular cases. The latter group was chosen because of our unanticipated finding showing that ocular only cases and DIF- ocular cases with multisite involvement had a lower proportion of cases with detectable pemphigoid autoantibodies. These results are illustrated in Figure 2 showing the test reactivity for the comparison of DIF- and DIF+ cases compared to controls, and in Figure 3 showing the test reactivity for the following different MMP phenotypes: ocular only, oral only, ocular and oral only, and all non-ocular sites of involvement.

Proportions of patients with positive serology with and without active inflammation and/or systemic immunosuppression

Supplementary Table 3 for patients with oral and/or ocular MMP (n=74) shows a strong association with disease activity but not with immunosuppression probably as 32/43 immunosuppressed patients (74.4%) still had active inflammation.

Proportions of DIF+ and DIF- cases with positive serum BM autoantibody reactivity for individual and test combinations: Figure 2 and Supplementary Expanded Table 2 show that, with 3 exceptions, compared to controls DIF+ cases have significantly different (more often positive) serology findings both for single tests and for all test combinations. DIF- cases with a positive BP230 ELISA, BP180-NC16a/IIF SSS combination or combinations of ELISA's/IIF SSS/laminin 332 assays were significantly different from controls (Figure 2) although the sensitivity is low for these tests (Figure 1)

Proportions of cases with ocular only, oral only, ocular and oral only and all non-ocular sites involved by MMP with positive serum BM autoantibody reactivity for individual tests and test combinations: In ocular only cases only 1/6 DIF+ cases had a positive serum test as opposed to 12/19 DIF+ non-ocular cases (Table 2) suggesting that there may be lower levels of detectable autoantibodies in ocular disease subjects independent of DIF status. Figure 3 and Supplementary Expanded Table 2 show that for ocular only MMP sites of involvement there was no significant difference in test reactivity compared to controls both for individual tests and for any test combinations. This finding was similar but less extreme for cases with both ocular and oral only involvement (n=15) for whom no individual test was significant. For all DIF- cases (n=24), a positive BP230 ELISA (4/24) was significantly different, as were test combinations including at least one positive ELISA and/or IIF SSS (6/24), in cases compared to controls. Conversely for pure oral, and any cases with non-ocular site involvement (all but one of which was DIF+) test reactivity was significantly different from controls for both ELISA's and for IgG SSS, as well as all test combinations.

Discussion

This cross-sectional study of 76 patients with a clinical diagnosis of MMP included 24 (32.9%) who were DIF- but who met clinical and pathology criteria for DIF- MMP with ocular involvement[3,18,22, 8,23] and included 18 with ocular only MMP (6/18 DIF+). To our knowledge this is the largest study of ocular only MMP studied to date.[2,15] Serum pemphigoid autoantibodies were detected in 29/76 (38.2%) of all MMP patients compared to 3/45 (6.7%) of controls in whom positive results were found only for ELISA's. The proportions of autoantibodies detected in DIF+ MMP was higher at 22/49 (44.9%) compared to DIF- MMP at 6/24 (25%). Lam 332 was positive in 3 DIF+ MMP cases. Serology was more often positive in patients with active inflammation.

Our primary hypothesis was that a panel of serum pemphigoid autoantibody tests might be used to confirm an immunopathological diagnosis of MMP in DIF- patients with ocular MMP involvement. All DIF- cases had ocular involvement. For DIF- cases the only serology test that was significantly different in cases compared to controls was that for BP230 reactivity (4/24). However, a test

combination including at least one positive ELISA and one positive IIF SSS increased the proportion of positive tests (6/24) and was significantly different from controls (see supplementary expanded Table 2 and Figure 2) but with low sensitivity (c.30%). For ocular only MMP (n=18) only 3/90 tests were positive; not significantly different from controls. In summary we have found only limited support for our primary hypothesis by finding that this panel of widely available serology tests do not contribute to the immunopathological diagnosis of ocular only MMP although they are of limited value in DIF- MMP multisite ocular disease; it is unsurprising that patients who don't have antibodies at the epithelial basement membrane (DIF negative), that are probably deposited from the circulation, are also less likely to have detectable circulating antibodies. Our findings for ocular only MMP confirm those of two other studies on a total of 16 patients. [2,15]

One potential shortcoming of this study might result from antibody degradation due to the storage methodology and the time between sample collection and analysis; we think this unlikely as antibody function in serum stored at -20C to -80C is both recommended for up to 10 years[24], has been shown to be stable for this period[25] and because our ELISAs were more often positive when duplicate sera were retested in Groningen and St John's 4-5 years after initial testing at the Service laboratory. Another shortcoming might relate to misclassification of our ocular only MMP cases; we think this unlikely given that the strict criteria we have used have recently become well established and coupled with the recognition that DIF and serology findings may be negative in ocular MMP.[3,8,18,22,23] Our serology results are compared with those of 13 similar MMP autoantibody studies in Supplementary Table 4a[2,13,16,26-35] and with 3 studies of control populations in Supplementary Table 4b[19,36,37]. Our findings for BP180 and BP230 ELISAs, Lam 332, IgA IIF SSS are comparable whereas our proportions of subjects having positive IgG IIF SSS are amongst the lowest reported. Differences in the proportions of routine tests that are positive relate both to differences in disease activity and in serum reactivity for MMP involving different anatomical sites , as we have shown in this study, with both quiescent disease and ocular sites having lower reactivity.

Strengths of this study are that it is a prospective hypothesis driven cross-sectional study for which subjects were diagnosed and phenotyped using previously agreed criteria and which utilized serology tests available in most dermatology immunopathology laboratories. Our results were duplicated in 2 independent laboratories and discrepancies verified in a third. Our finding of 51/468 (10.9%) discrepancies for duplicate testing, of which only 6 from one laboratory could be confirmed, shows that interpretation of results requires confidence in the quality standards of the laboratory being used. It is also unique (Supp Table 4) in including, at the time of blood sampling; disease activity scores, immunosuppression data; a control population; and serum storage data.

The findings from this study on the value of circulating autoantibody tests for the immunopathological diagnosis of MMP concur with those of previous studies on the poor sensitivity of DIF in MMP with

ocular involvement and the need for an alternative diagnostic strategy for ocular disease. Given the low sensitivity of serology tests in MMP and the false positive rate in controls, the finding of a positive result must be interpreted with caution before using these as confirmation of a diagnosis of MMP. Our recommendation for a diagnostic protocol for ocular MMP arising from these studies is in Figure 4.[33,38-40] Our studies also have implications for the development of diagnostic tests and for the pathogenesis of MMP. Either current immunopathology tests are too insensitive for the detection of low levels of tissue fixed or circulating antibodies or there is a subset of MMP patients in whom an alternative, possibly cell mediated, immunopathology directed at the epithelial basement membrane epitopes is predominant.[8] Novel tests for MMP are required that might include cellular, cytokine or gene expression biomarkers for MMP.

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Figure

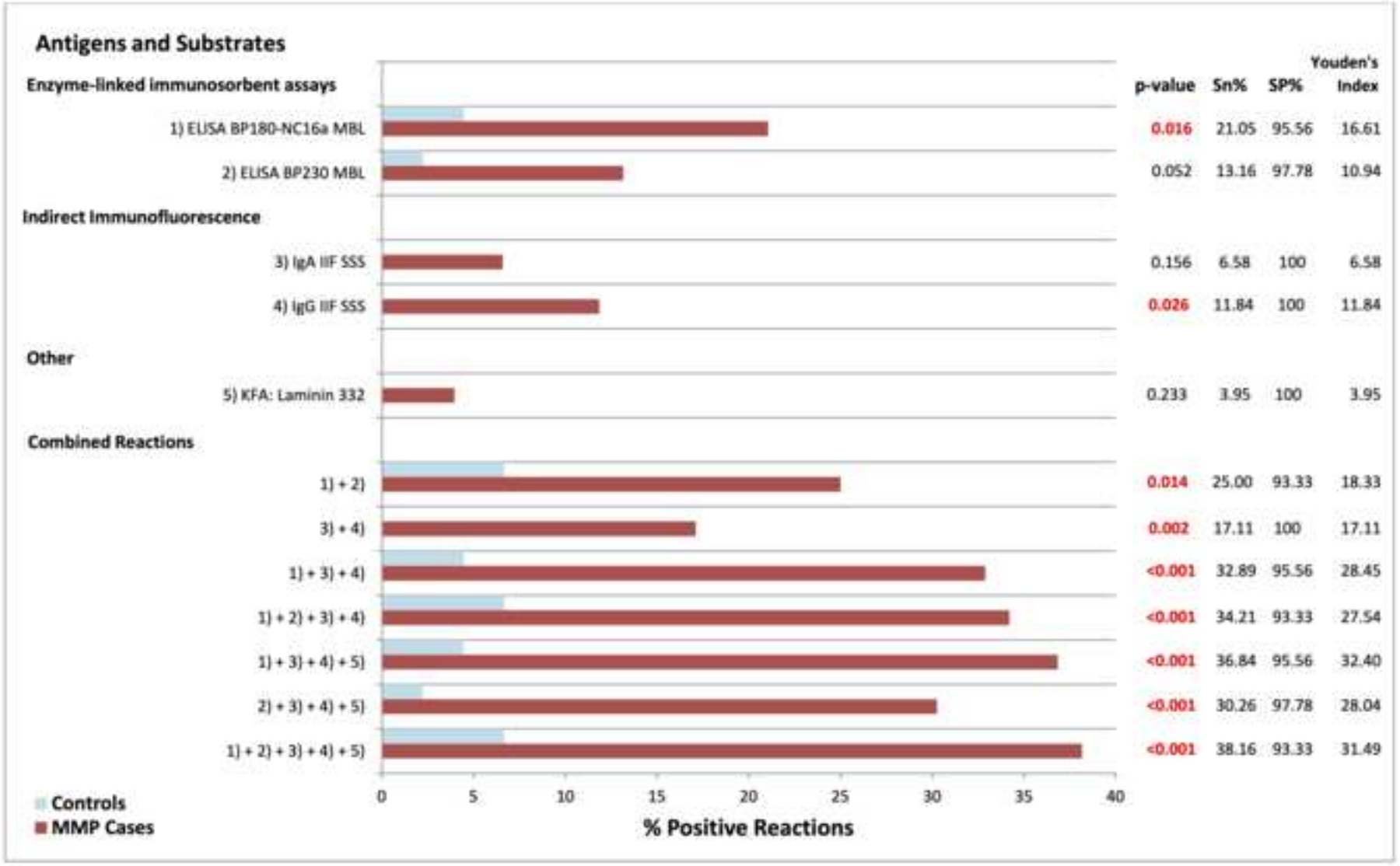


Figure 2

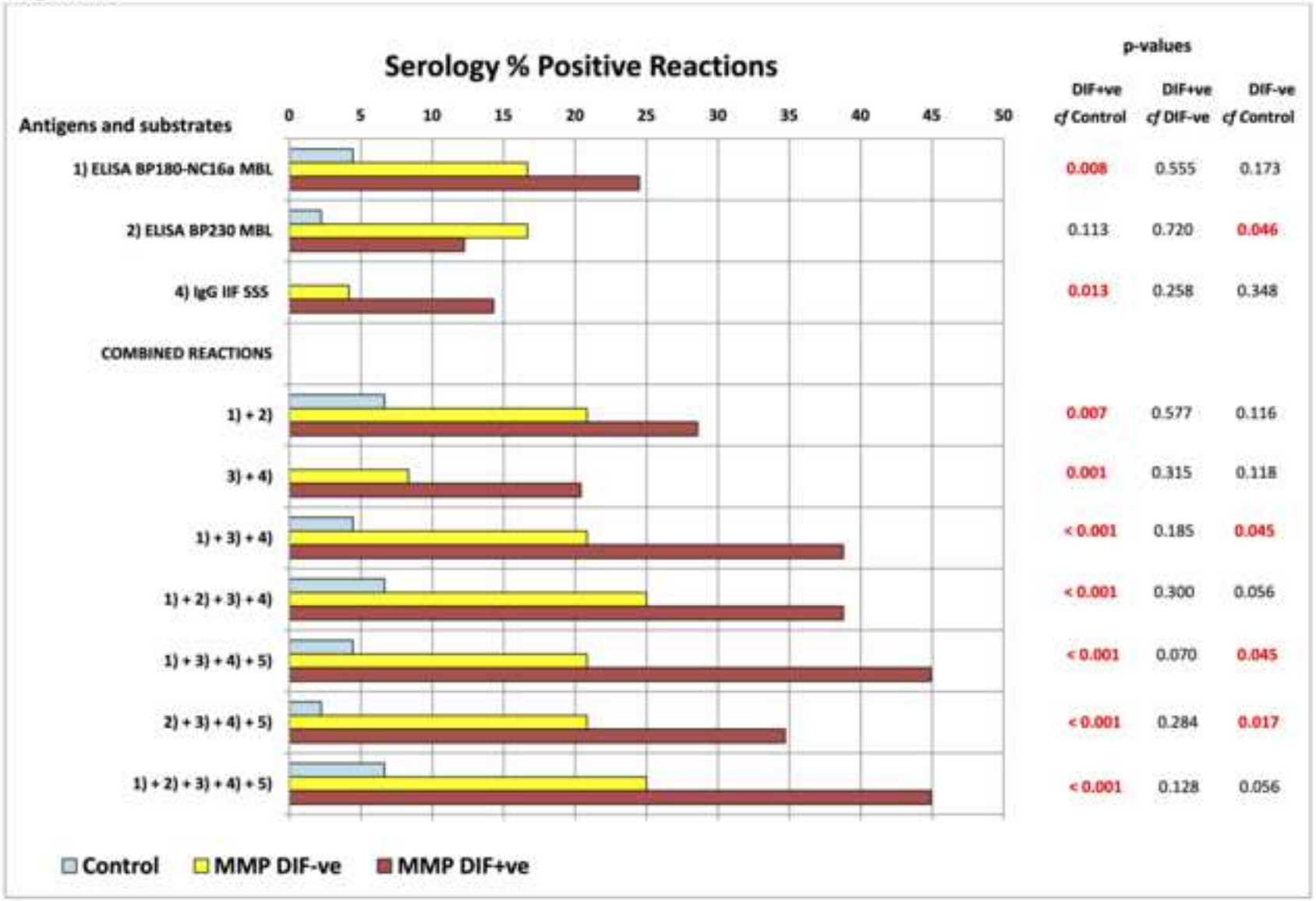
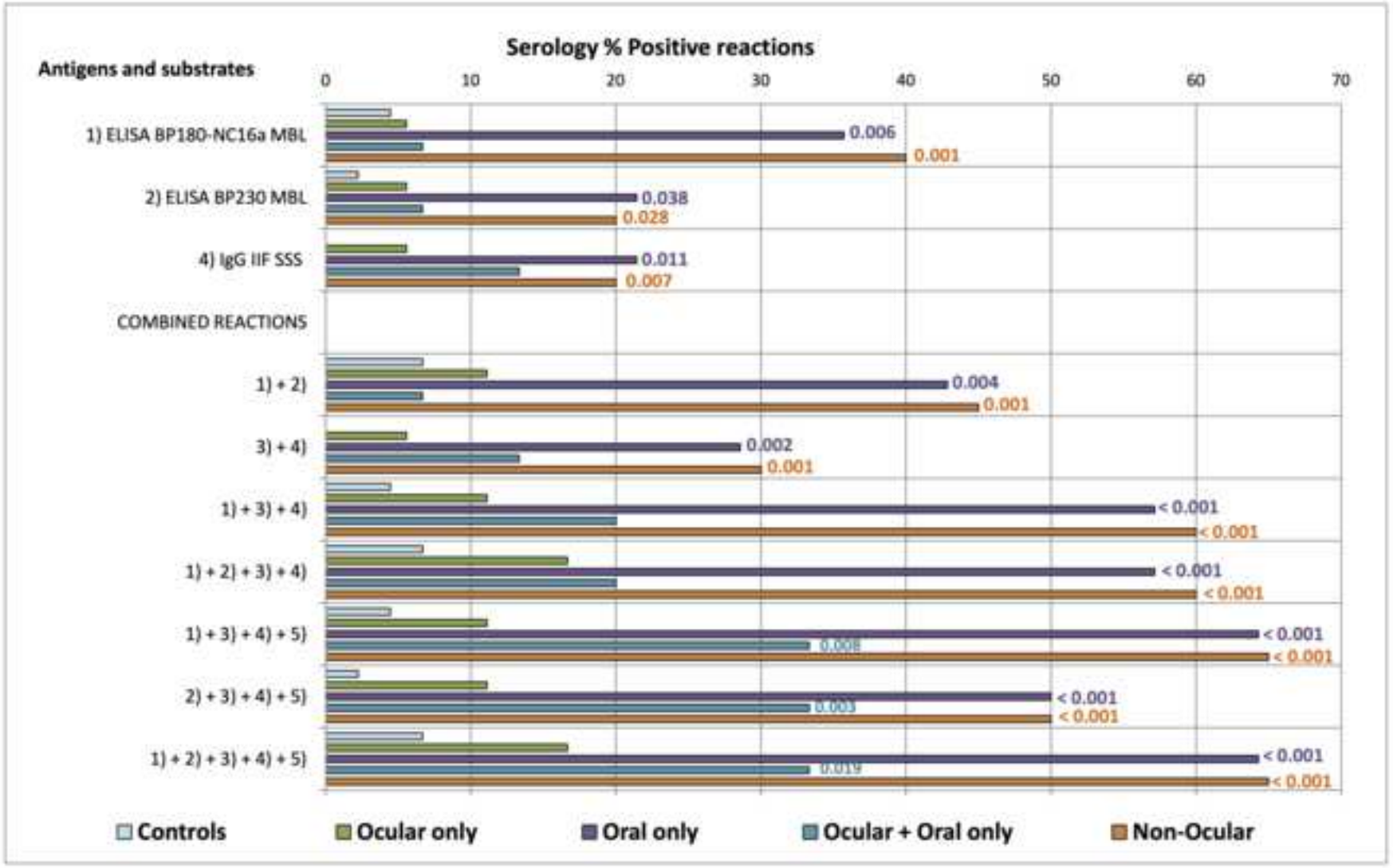


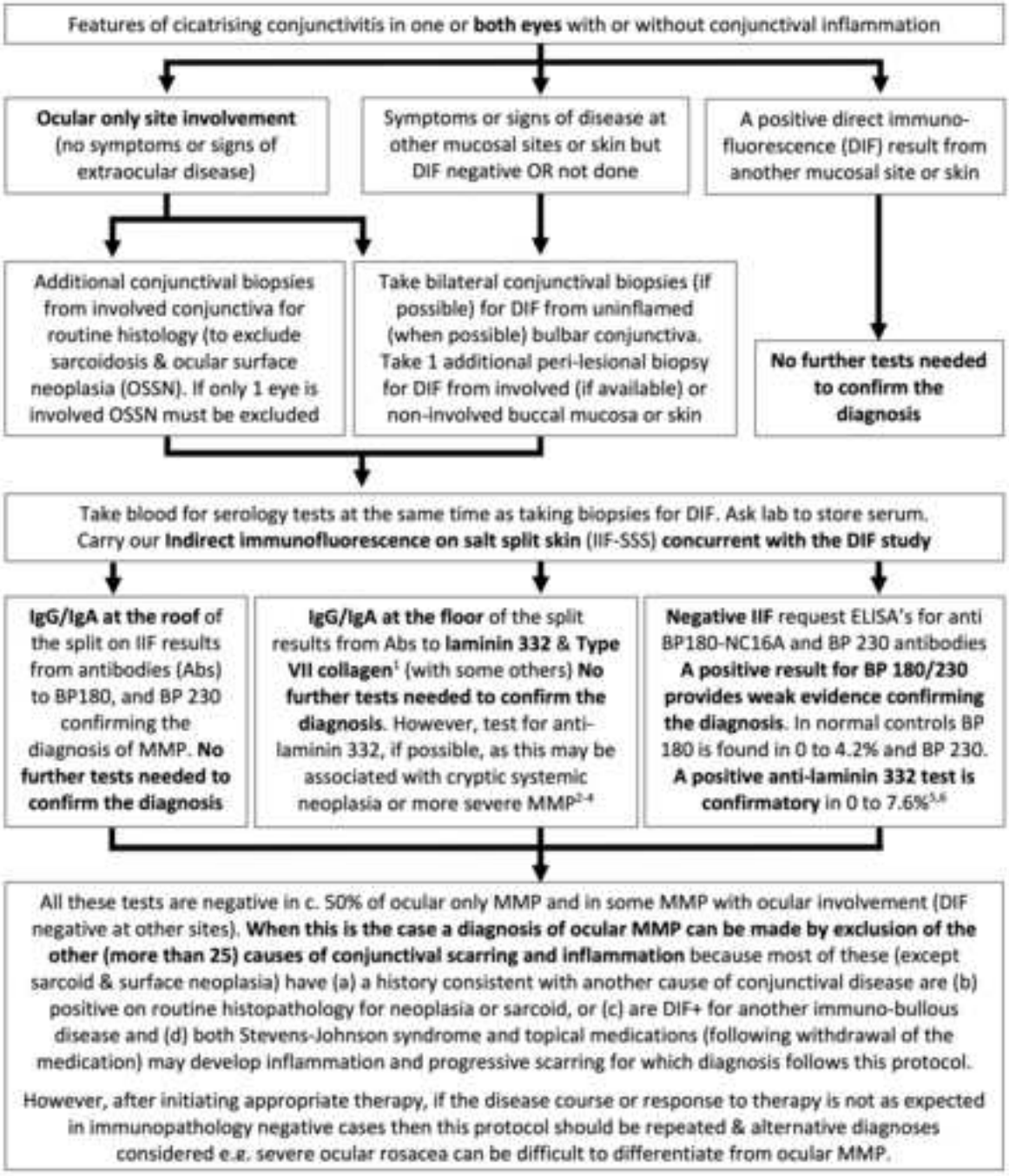
Figure 3



Figure

Click here to access/download;Figure;Figure 4 Diagnostic protocol_3_7_20TIFF.tiff

Figure 4



Footnotes

1. Anti-type V11 collagen reactivity is indicative of acquired epidermolysis bullosa acquisita (EBA) which may cause more severe extraocular MMP but does not alter therapy for the ocular component.
2. Egan CA, Lazarova Z, Darling TN, et al. Anti-epiligrin cicatricial pemphigoid: clinical findings, immunopathogenesis, and significant associations. Medicine (Baltimore) 2003;82(3):177-86.
3. Matsushima S, Horiguchi Y, Honda T, et al. A case of anti-epiligrin cicatricial pemphigoid associated with lung carcinoma and severe laryngeal stenosis: review of Japanese cases and evaluation of risk for internal malignancy. J Dermatol 2004;31(1):10-5.
4. Bernard P, Antonicelli F, Bedane C, et al. Prevalence and clinical significance of anti-laminin 332 autoantibodies detected by a novel enzyme-linked immunosorbent assay in mucous membrane pemphigoid. JAMA Dermatol 2013;149(5):533-40.
5. See Supp Table 3b. Anti-laminin 332 antibodies are very uncommon in controls (<1%).
6. Goletz S, Probst C, Komorowski L, et al. A sensitive and specific assay for the serological diagnosis of antilaminin 332 mucous membrane pemphigoid. The British journal of dermatology 2019;180(1):149-56. Until recently anti-Lam 332 antibody tests have only been available in specialised labs. This test is now commercially available and should make anti-laminin 332 antibody testing more widely available: https://www.euroimmun.com/suche.html

Table

Table 1 Description of the 5 tests used to detect serum pemphigoid autoantibodies in mucous membrane pemphigoid (MMP) patients, the proportions of positive results compared to controls, sensitivity, specificity and Youden's index. All p-values are exact (2-sided).

Test n #	Antigens and substrates	Test methodology*†	Positive Reactions n (%)		Exact p-value	Sensitivity & Specificity		
			MMP Cases total n=76	Controls total n=45		Sn%	Sp%	Youden's index
		Enzyme-linked immunosorbent assays						
1)	ELISA BP180-NC16a MBL	Medical & Biological Laboratories (MBL) International®. Cut-off < 9 U/ml	16 (21.05)	2 (4.44)	**0.016**	21.05	95.56	16.61
2)	ELISA BP230 MBL		10 (13.16)	1 (2.22)	0.052	13.16	97.78	10.94
		Indirect Immunofluorescence						
3)	IgA IIF SSS	Indirect immunofluorescence on human 1 molar salt split skin (SSS)	5 (6.58)	0 (0.00)	0.156	6.58	100	6.58
4)	IgG IIF SSS		9 (11.84)	0 (0.00)	**0.026**	11.84	100	11.84
		Laminin 332 (Lam 332) assays						
5)	Keratinocyte footprint assay (KFA)	KFA (Groningen) for Lam 332 antibody detection	3 (3.95)	0 (0.00)	0.233	3.95	100	3.95
Combined Reactions								
1) + 2)			19 (25.00)	3 (6.67)	**0.014**	25.00	93.33	18.33
3) + 4)			13 (17.11)	0 (0.00)	**0.002**	17.11	100	17.11
1) + 3) + 4)			25 (32.89)	2 (4.44)	**< 0.001**	32.89	95.56	28.45
1) + 2) + 3) + 4)			26 (34.21)	3 (6.67)	**< 0.001**	34.21	93.33	27.54
1) + 3) + 4) + 5)			28 (36.84)	2 (4.44)	**< 0.001**	36.84	95.56	32.40
2) + 3) + 4) + 5)			23 (30.26)	1 (2.22)	**< 0.001**	30.26	97.78	28.04
1) + 2) + 3) + 4) + 5)			29 (38.16)	3 (6.67)	**< 0.001**	38.16	93.33	31.49

#Test numbering is used in Figures 1-3.

*All tests were on 76 cases and 45 controls apart from BP230 ELISA which were performed on only 60/76 MMP cases at the Service laboratory.

† Commercially available tests were carried out according to the manufacturer's instructions. Indirect immunofluorescence was carried out as previously described.[31]

Table

Table 2. Clinical characteristics of mucous membrane pemphigoid (MMP) cases and controls, direct immunofluorescence results, and serum pemphigoid autoantibody test results for all cases, and cases with both limited site and multiple site involvement. Non-ocular, nasopharyngeal, genital and skin categories are not mutually exclusive.

DEMOGRAPHICS	**ALL MMP**	**MMP cases categorized by different sites of involvement: non-ocular, nasopharyngeal, genital and skin categories are not mutually exclusive**							**CONTROLS**
		Ocular only	**Ocular & oral only**	**Oral only**	**All Non-ocular**	**Nasopharyngeal & any other**	**Genital & any other**	**Skin & any other**	
Number (%)	76	18	15	14	20	16	8	14	45
Male	38 (50%)	9 (50%)	11 (73.3%)	5 (35.7%)	5 (25.0%)	6 (37.5%)	2 (25.0%)	8 (57.1%)	22 (49%)
Age: Mean [standard deviation] Range	59.9 [14.6] 18-83	63.2 [18.0] 24-83	55.4 [10.5] 38-75	61.1 [10.0] 47-81	63.7 [9.5] 47-81	57.4 [17.7] 18-78	66.25 [7.8] 56-76	57.4 [15.2] 23-74	61.4 [13.1] 18-86
White race*	64 (91.4%)	16 (88.9%)	13 (100%)	11 (91.7%)	15 (83.3%)	14 (93.3%)	5 (71.4%)	11 (84.6)	42 (93%)
Race not declared#	6	0	2	2	3	1	1	1	0
Systemic immunotherapy	43 (56.6%)	13 (72.2%)	9 (60.0%)	3 (21.4%)	5 (25.0%)	12 (75.0%)	3 (37.5%)	8 (57.1%)	None
Direct immunofluorescence results (DIF)									
DIF positive	49 (67.1%)	6 (35.3%)	12 (80%)	13 (100%)	19 (100%)	11 (68.7%)	4 (57.1%)	9 (64.3%)	Not done
DIF negative (all ocular)†	24 (32.9%)	11 (64.7%)	3 (20%)	0 (0.0%)	0 (0.0%)	5 (31.3%)	3 (42.9%)	5 (35.7%)	
DIF unknown#	3	1	0	1	1	0	1	0	
Serum autoantibody (SA) results: n (%)									
Any positive	29 (38.2)	3 (16.7)	5 (33.3)	9 (64.3)	13 (65.0)	7 (43.8)	6 (75.0)	5 (35.7)	3 (6.7)
SA positive in DIF positive	22/49 (44.9)	1/6 (16.7)	5/12 (41.7)	8/13 (61.5)	12/19 (63.2)	5/11 (45.5)	3/4 (75.0)	3/9 (33.3)	Not Applicable
SA positive in DIF negative	6/24 (25.0)	2/11 (18.2)	0/3 (00.0)	0/0	0/0	2/5 (40.0)	3/3 (100.0)	2/5 (40.0)	

*Numbers and percentages are for white races: MMP cases additionally included 2 Asians, 1 Black and 3 Other races, Controls additionally included 2 Asians and 1 Black.
#Missing values for Race and DIF are shown: these were excluded from the denominators for calculation of percentages.
†DIF negative patients all had ocular involvement: 11/24 (45.8%) were ocular only, 3/24 (12.5%) had ocular and oral involvement only, the remaining 10/24 (41.6%) had ocular involvement with the other non-ocular sites (nasopharyngeal, genital and skin).

Supplemental Table (online only)

Supplementary Table 1 Dataset for individual patients and controls including phenotyping data, and results of serum pemphigoid autoantibody tests

MMP Site; MMP History

Patient study ID	Direct IF result	Site NOT Screened	MMP Site Positive at Screening	Non-ocular MMP	Age at diagnosis	sex	Ethnicity	Follow-up (months)
001	Negative	All Screened	ocular oral Nasopharynx	none	71	F	White-British	133.42
002	Positive	oral skin nasophx genital perianal	ocular	none	77	F	White-British	74.60
003	Negative	All Screened	ocular	none	61	M	White-British	54.42
004	Negative	oral skin nasophx genital perianal	ocular	none	74	M	White-British	54.42
005	Positive	nasophx	ocular oral	oral	58	M	White-Other	52.42
006	Negative	oral skin nasophx genital perianal	ocular	none	49	F	White-British	345.42
007	Positive	nasophx	ocular oral	oral	53	F	White-British	256.42
008	Positive	All Screened	ocular oral skin Nasopharynx	oral oesophageal	60	M	White-British	37.42
009	Positive	oral nasophx genital perianal	ocular skin	oral	55	M	White-British	64.85
010	Negative	All Screened	ocular oral Nasopharynx	none	56	F	White-British	109.42
011	Negative	All Screened	ocular	oral skin	61	M	Other ethnic	103.92
013	Positive	All Screened	ocular oral	oral	63	M	White-British	57.42
014	Positive	All Screened	ocular	none	64	F	White-Other	232.42
015	Negative	nasophx	ocular	drug-related	45	M	White-Other	230.42
016	Positive	nasophx	ocular oral skin	oral skin	74	M	White-British	68.42
017	Positive	oral skin nasophx genital perianal	ocular	none	82	M	White-British	73.42
018	Positive	oral skin nasophx genital perianal	none/unknown	oral	50	F	White-British	50.42
019	Negative	nasophx	ocular	none	24	F	Black African	56.42
020	Positive	nasophx	ocular oral	none	54	M	White-British	141.42
021	Positive	nasophx	ocular oral	oral	58	M	White-British	62.42
022	Positive	nasophx	ocular oral	none	64	F	White-Irish	185.42
023	Negative	nasophx	ocular oral skin	oral nasopharyngeal oesophageal	57	F	White-British	175.42
024	Negative	nasophx	ocular oral genital	oral	73	M	White-British	121.42
025	Negative	All Screened	ocular oral	oral	51	M	White-British	132.42
026	Negative	oral skin nasophx genital perianal	ocular	none	82	M	White-British	181.42
027	Positive	oral skin nasophx genital perianal	ocular	oral nasopharyngeal	41	M	White-British	238.42
028	Positive	nasophx	ocular	oral	39	M	Not stated	166.99
029	Positive	nasophx	ocular oral	oral genital	60	F	White-British	120.99
030	Unknown	perianal	ocular genital	none	70	M	White-British	139.49
031	Positive	nasophx	oral	oral nasopharyngeal genital rectal	74	F	Not stated	60.99
032	Negative	nasophx	ocular oral	oral nasopharyngeal genital	60	F	Other ethnic	126.99
034	Positive	nasophx	ocular oral	none	75	M	White-British	60.99
035	Positive	All Screened	oral	oral	75	F	Other ethnic	36.42
036	Positive	All Screened	oral	oral	64	F	White-British	157.42
037	Negative	nasophx	ocular oral skin	oral	23	M	White-British	301.42
038	Positive	nasophx	ocular oral genital	oral	76	F	White-British	147.42
039	Negative	All Screened	ocular	none	74	M	White-British	125.53
040	Positive	All Screened	ocular oral skin Nasopharynx	oral nasopharyngeal	57	M	White-British	63.42
041	Negative	All Screened	ocular	none	68	M	Indian	41.42
042	Positive	nasophx	ocular oral	oral	38	M	White-British	98.42
043	Negative	nasophx	ocular	oral	43	F	White-British	71.42
044	Negative	nasophx	ocular	none	76	F	White-British	78.42
045	Positive	All Screened	ocular	none	46	F	White-Irish	141.42
048	Positive	nasophx	oral	oral	57	M	White-British	122.42
049	Positive	All Screened	ocular	oral	60	M	White-British	59.42
050	Positive	nasophx	none/unknown	skin genital	61	F	Other Asian	56.42
051	Positive	All Screened	ocular oral	oral	53	F	Not stated	87.42
052	Positive	All Screened	oral	oral	47	F	White-British	48.42
053	Negative	oral skin nasophx genital perianal	ocular	none	29	M	White-British	217.16
054	Positive	All Screened	ocular oral Nasopharynx	oral oesophageal	40	F	White-British	325.42
055	Negative	All Screened	ocular skin	none	68	M	White-British	69.42
056	Negative	All Screened	ocular	none	59	F	White-British	139.42
057	Unknown	nasophx	oral	oral	73	F	White-British	37.42
058	Positive	All Screened	oral Nasopharynx	oral nasopharyngeal	69	F	White-British	36.42
059	Positive	All Screened	oral	oral	66	M	White-British	169.42
060	Positive	All Screened	oral	oral	63	M	Not stated	41.42
062	Positive	All Screened	ocular Nasopharynx	none	68	M	White-British	109.42
065	Positive	All Screened	ocular	none	83	F	White-British	29.42
066	Negative	All Screened	ocular oral	none	70	M	White-British	145.42
068	Positive	All Screened	oral	oral	61	M	Not stated	140.42
069	Positive	All Screened	oral	oral	57	F	White-British	35.42
071	Negative	nasophx	ocular oral skin	oral skin genital	56	F	White-British	260.42
072	Positive	oral skin nasophx genital perianal	ocular	oral skin	62	F	Not stated	43.42
073	Positive	All Screened	oral	oral	81	F	White-British	38.42
074	Positive	skin nasophx genital perianal	oral	oral	56	M	White-British	169.42
076	Positive	All Screened	oral	oral	51	F	White-British	97.42
077	Positive	All Screened	oral Nasopharynx	oral	71	F	White-British	53.42
078	Positive	All Screened	oral	oral	55	F	White-British	26.42
079	Positive	All Screened	oral skin	oral skin	70	F	White-British	43.42
081	Positive	All Screened	ocular oral skin Nasopharynx	oral skin	26	M	White-British	26.42
082	Positive	All Screened	oral skin Nasopharynx	oral skin	73	F	White-British	43.42
084	Positive	All Screened	ocular oral	oral	52	M	White-British	132.42
096	Positive	All Screened	ocular oral Nasopharynx	oral oesophageal	18	M	White-British	301.72
099	Negative	All Screened	ocular Nasopharynx	oral nasopharyngeal	78	F	White-British	27.42
102	Positive	oral skin nasophx genital perianal	ocular	none	78	F	White-British	33.42
107	Uncertain	oral skin genital perianal	ocular	none	67	M	White-British	22.42

MMP Site of Involvement at Screening; Systemic Immunotherapy; Ocular Disease at Screening

Patient study ID	Ocular	Oral	Skin	Nasopharyngeal	Genital	Perianal	Other autoimmune disease or Cancer	Drug code	Treatment	Total Inflammation ≥ 5 (both eyes)	History of Entropion surgery or Fornix reconstruction
001	POSITIVE	POSITIVE	Negative	POSITIVE	Negative	Negative	none	2	YES	YES	YES
002	POSITIVE	Declined	Declined	Declined	Declined	Declined	none	2	YES	YES	YES
003	POSITIVE	Negative	Negative	Negative	Negative	Negative	YES	2	YES	no	YES
004	POSITIVE	Declined	Declined	Declined	Declined	Declined	YES	0	none	no	YES
005	POSITIVE	POSITIVE	Negative	Uncertain	Negative	Negative	none	2, 6	YES	no	no
006	POSITIVE	Declined	Declined	Declined	Declined	Declined	YES	3	YES	no	YES
007	POSITIVE	POSITIVE	Negative	Declined	Negative	Negative	none	0	none	YES	YES
008	POSITIVE	POSITIVE	POSITIVE	POSITIVE	Negative	Negative	none	0	none	no	no
009	POSITIVE	Unknown	POSITIVE	Uncertain	Unknown	Unknown	none	4	YES	no	YES
010	POSITIVE	POSITIVE	Negative	POSITIVE	Negative	Negative	none	2	YES	YES	YES
011	POSITIVE	Negative	Negative	Negative	Negative	Negative	YES	3	YES	YES	YES
013	POSITIVE	POSITIVE	Negative	Negative	Negative	Negative	YES	4	YES	no	no
014	POSITIVE	Negative	Negative	Negative	Negative	Negative	YES	4	YES	YES	YES
015	POSITIVE	Negative	Negative	Declined	Negative	Negative	none	2	YES	YES	YES
016	POSITIVE	POSITIVE	POSITIVE	Declined	Negative	Negative	none	2, 4	YES	YES	YES
017	POSITIVE	Declined	Declined	Declined	Declined	Declined	YES	4	YES	no	YES
018	Negative	Declined	Declined	Declined	Declined	Declined	none	0	none	N/A no ocular MMP	no
019	POSITIVE	Negative	Negative	Declined	Negative	Negative	none	2	YES	YES	no
020	POSITIVE	POSITIVE	Negative	Declined	Negative	Negative	none	2, 4	YES	YES	YES
021	POSITIVE	POSITIVE	Negative	Declined	Negative	Negative	YES	9	YES	YES	YES
022	POSITIVE	POSITIVE	Negative	Declined	Negative	Negative	YES	4, 5	YES	no	YES
023	POSITIVE	POSITIVE	POSITIVE	Declined	Negative	Negative	none	2, 4, 6	YES	no	YES
024	POSITIVE	POSITIVE	Negative	Declined	POSITIVE	Negative	none	0	none	YES	YES
025	POSITIVE	POSITIVE	Negative	Negative	Negative	Negative	none	0	none	no	no
026	POSITIVE	Declined	Declined	Declined	Declined	Declined	none	9	YES	no	YES
027	POSITIVE	Declined	Declined	Declined	Declined	Declined	none	2, 4	YES	YES	YES
028	POSITIVE	Negative	Negative	Declined	Negative	Negative	none	0	none	no	no
029	POSITIVE	POSITIVE	Negative	Declined	Negative	Negative	none	2,4	YES	no	YES
030	POSITIVE	Negative	Negative	Negative	POSITIVE	Unknown	YES	0	none	no	no
031	Negative	POSITIVE	Negative	Declined	Negative	Negative	YES	0	none	N/A no ocular MMP	no
032	POSITIVE	POSITIVE	Negative	Declined	Negative	Negative	YES	3	YES	no	YES
034	POSITIVE	POSITIVE	Negative	Declined	Negative	Negative	YES	2, 4	YES	no	YES
035	Negative	POSITIVE	Negative	Negative	Negative	Negative	YES	0	none	N/A no ocular MMP	no
036	Negative	POSITIVE	Negative	Negative	Negative	Negative	YES	0	none	N/A no ocular MMP	YES
037	POSITIVE	POSITIVE	POSITIVE	Declined	Negative	Negative	YES	2, 4	YES	YES	YES
038	POSITIVE	POSITIVE	Negative	Uncertain	POSITIVE	Negative	YES	0	none	no	YES
039	POSITIVE	Negative	Negative	Negative	Negative	Negative	none	2	YES	YES	YES
040	POSITIVE	POSITIVE	POSITIVE	POSITIVE	Negative	Negative	none	0	none	no	YES
041	POSITIVE	Negative	Negative	Negative	Negative	Negative	none	0	none	no	YES
042	POSITIVE	POSITIVE	Negative	Unknown	Negative	Negative	none	6	YES	no	no
043	POSITIVE	Negative	Negative	Declined	Negative	Negative	YES	0	none	no	no
044	POSITIVE	Negative	Negative	Unknown	Negative	Negative	none	4	YES	no	YES
045	POSITIVE	Negative	Negative	Negative	Negative	Negative	YES	2	YES	YES	YES
048	Negative	POSITIVE	Negative	Declined	Negative	Negative	none	4	YES	N/A no ocular MMP	no
049	POSITIVE	Negative	Negative	Negative	Negative	Negative	none	0	none	no	YES
050	Negative	Negative	Negative	Declined	Negative	Negative	none	0	none	N/A no ocular MMP	YES
051	POSITIVE	POSITIVE	Negative	Negative	Negative	Negative	none	4	YES	no	YES
052	Negative	POSITIVE	Negative	Negative	Negative	Negative	none	0	none	N/A no ocular MMP	no
053	POSITIVE	Declined	Declined	Uncertain	Declined	Declined	YES	4	YES	no	no
054	POSITIVE	POSITIVE	Negative	POSITIVE	Negative	Negative	none	5	YES	YES	YES
055	POSITIVE	Negative	POSITIVE	Negative	Negative	Negative	none	0	none	no	YES
056	POSITIVE	Negative	Negative	Negative	Negative	Negative	none	0	none	YES	YES
057	Negative	POSITIVE	Negative	Uncertain	Negative	Negative	YES	0	none	N/A no ocular MMP	no
058	Negative	POSITIVE	Negative	POSITIVE	Negative	Negative	none	0	none	N/A no ocular MMP	no
059	Negative	POSITIVE	Negative	Negative	Negative	Negative	none	0	none	N/A no ocular MMP	no
060	Negative	POSITIVE	Negative	Negative	Negative	Negative	YES	0	none	N/A no ocular MMP	YES
062	POSITIVE	Negative	Negative	POSITIVE	Negative	Negative	YES	2	YES	no	YES
065	POSITIVE	Negative	Negative	Negative	Negative	Negative	none	0	none	no	YES
066	POSITIVE	POSITIVE	Negative	Negative	Negative	Negative	none	0	none	YES	YES
068	Negative	POSITIVE	Negative	Negative	Negative	Negative	none	0	none	N/A no ocular MMP	YES
069	Negative	POSITIVE	Negative	Negative	Negative	Negative	none	0	none	N/A no ocular MMP	no
071	POSITIVE	POSITIVE	POSITIVE	Declined	Negative	Negative	none	2, 5	YES	YES	YES
072	POSITIVE	Declined	Declined	Declined	Declined	Declined	YES	0	none	no	no
073	Negative	POSITIVE	Negative	Negative	Negative	Negative	YES	2	YES	N/A no ocular MMP	no
074	Negative	POSITIVE	Declined	Declined	Declined	Declined	none	4	YES	N/A no ocular MMP	no
076	Negative	POSITIVE	Negative	Negative	Negative	Negative	YES	0	none	N/A no ocular MMP	no
077	Negative	POSITIVE	Negative	POSITIVE	Negative	Negative	none	4	YES	N/A no ocular MMP	no
078	Negative	POSITIVE	Negative	Negative	Negative	Negative	none	0	none	N/A no ocular MMP	no
079	Negative	POSITIVE	POSITIVE	Negative	Negative	Negative	none	0	none	N/A no ocular MMP	no
081	POSITIVE	POSITIVE	POSITIVE	POSITIVE	Negative	Negative	YES	6,8,9	YES	no	no
082	Negative	POSITIVE	POSITIVE	POSITIVE	Negative	Negative	YES	5,6,9	YES	N/A no ocular MMP	YES
084	POSITIVE	POSITIVE	Negative	Negative	Negative	Negative	none	4	YES	no	no
096	POSITIVE	POSITIVE	Negative	POSITIVE	Negative	Negative	YES	4,6	YES	no	no
099	POSITIVE	Negative	Negative	POSITIVE	Negative	Negative	none	2	YES	no	YES
102	POSITIVE	Declined	Declined	Declined	Declined	Declined	none	2	YES	YES	YES
107	POSITIVE	Declined	Declined	Negative	Declined	Declined	YES	0	none	YES	YES

Ocular Disease at Screening (continued); Oral disease inflammatory activity; Classification

Patient study ID	Tauber Stage Right eye	Tauber Stage Left eye	Tauber Stage Worst eye > IIb or >IIIb	Central corneal conditions 3, 5, 6, 8 *	Score	Score > 0	Classification by of site of involvement by ocular only, oral only, ocular and oral only, non-ocular and ocular with other mixed sites for Expanded Table 2	Classification by main site of involvement***
001	IIc, IIIa (4)	IId, IIIa (1)	YES	PRESENT	1	YES	5:Other Ocular/mix	Nasopharyngeal ± other site (definitive clinical)
002	IIb, IIIa (2)	IIb, IIIa (2)	no	PRESENT	DS	DS / Unknown	1:Ocular only	Ocular only (probable clinical)
003	IIa, IIIa (2)	IIa, IIIa (1)	no	absent	0	N/A no oral MMP	1:Ocular only	Ocular only (definitive clinical)
004	lla, llla (3)	IIa, IIIa (1)	no	absent	DS	DS / Unknown	1:Ocular only	Ocular only (probable clinical)
005	IIa, IIIa (1)	I	no	absent	4	YES	3:Ocular+Oral only	Ocular & Oral only (probable clinical)
006	IId, IIId (4)	0	YES	absent	DS	DS / Unknown	1:Ocular only	Ocular only (probable clinical)
007	IIa	IIa	no	absent	12	YES	3:Ocular+Oral only	Ocular & Oral only (probable clinical)
008	0	1	no	absent	12	YES	5:Other Ocular/mix	Skin and Nasopharyngeal ± other site (definitive clinical)
009	IIb, IIIc,(4)	IIb, IIIc (3)	YES	absent	-9999	DS / Unknown	5:Other Ocular/mix	Skin ± other site (definitive clinical)
010	IIb, IIIb (4)	IIa, IIIa (3)	no	PRESENT	12	YES	5:Other Ocular/mix	Nasopharyngeal ± other site (definitive clinical)
011	IIb, IIIa (1)	IIb, IIIa (1)	no	absent	0	no	5:Other Ocular/mix	Skin ± other site (definitive clinical)
013	IIa, IIa (1)	IIa, IIIa (1)	no	absent	8	YES	3:Ocular+Oral only	Ocular & Oral only (definitive clinical)
014	IId, IIId (4)	IIa, IIIa	YES	PRESENT	0	N/A no oral MMP	1:Ocular only	Ocular only (definitive clinical)
015	IIb, IIb (2)	IIb, IIIc (2)	YES	PRESENT	0	N/A no oral MMP	1:Ocular only	Ocular only (definitive clinical)
016	IIc, III (3)	IIc, IIIb (2)	YES	absent	8	YES	5:Other Ocular/mix	Skin ± other site (definitive clinical)
017	IId, IIIc (2)	IId, IIIc (2)	YES	absent	DS	DS / Unknown	1:Ocular only	Ocular only (probable clinical)
018	0	0	N/A	N/A no ocular MMP	DS	DS / Unknown	2:Oral only	Oral only (probable clinical)
019	IIa, IIIb (2)	IIa, IIIb (2)	no	PRESENT	0	N/A no oral MMP	1:Ocular only	Ocular only (probable clinical)
020	IId, IIId (2)	IIa, IIIa	YES	PRESENT	19	YES	3:Ocular+Oral only	Ocular & Oral only (probable clinical)
021	IIa	IId, IIIc (4)	YES	absent	10	YES	3:Ocular+Oral only	Ocular & Oral only (probable clinical)
022	llb, llla (1)	llc, lllb (2)	YES	PRESENT	1	YES	3:Ocular+Oral only	Ocular & Oral only (probable clinical)
023	IId, IIIa (2)	IIc, IIIb (2)	YES	absent	36	YES	5:Other Ocular/mix	Skin & nasopharyngeal ± other site (definitive clinical)
024	IIb, IIIc (2)	IIc, IIIb (2)	YES	absent	16	YES	5:Other Ocular/mix	Nasopharyngeal ± other site (DS/Uncertain)
025	IIa	0	no	absent	12	YES	3:Ocular+Oral only	Ocular & Oral only (definitive clinical)
026	IId, IIId (4)	I	YES	PRESENT	DS	DS / Unknown	1:Ocular only	Ocular only (probable clinical)
027	IIc, IIIc (2)	IId, IIId (2)	YES	absent	DS	DS / Unknown	5:Other Ocular/mix	Nasopharyngeal ± other site (definitive clinical)
028	IIa, IIIa	IIa, IIIa	no	absent	0	no	3:Ocular+Oral only	Ocular & Oral only (probable clinical)
029	IId, IIIc	IId, IIIc	YES	absent	17	YES	5:Other Ocular/mix	Nasopharyngeal ± other site (DS/Uncertain)
030	IId, IIId	-8888	YES	PRESENT	0	N/A no oral MMP	5:Other Ocular/mix	Other mixed [ocular and genital] sites (definitive clinical)
031	I	I	N/A	N/A no ocular MMP	16	YES	4:Other non-ocular	Nasopharyngeal ± other site (definitive clinical)
032	IIa, IIIa	IIb, IIIc	YES	absent	5	YES	5:Other Ocular/mix	Nasopharyngeal ± other site (definitive clinical)
034	IIa	IIa, IIIa (1)	no	absent	30	YES	3:Ocular+Oral only	Ocular & Oral only (probable clinical)
035	0	0	N/A	N/A no ocular MMP	14	YES	2:Oral only	Oral only (definitive clinical)
036	0	0	N/A	N/A no ocular MMP	15	YES	2:Oral only	Oral only (definitive clinical)
037	IIa, IIIa	IIc, IIIa	YES	absent	17	YES	5:Other Ocular/mix	Skin ± other site (definitive clinical)
038	IId, IIIb	IId, IIIc	YES	absent	4	YES	5:Other Ocular/mix	Nasopharyngeal ± other site (DS/Uncertain)
039	IIa, IIIb (2)	IIa, IIIb (2)	no	absent	0	N/A no oral MMP	1:Ocular only	Ocular only (definitive clinical)
040	IIc, IIIc (3)	IIc, IIIb (1)	YES	absent	0	no	5:Other Ocular/mix	Skin & nasopharyngeal ± other site (definitive clinical)
041	IId, IIId	IId, IIId	YES	absent	0	N/A no oral MMP	1:Ocular only	Ocular only (definitive clinical)
042	I	0	no	absent	6	YES	3:Ocular+Oral only	Ocular & Oral only (probable clinical)
043	IIb, IIIa (2)	IIb, IIIa (2)	no	absent	0	no	3:Ocular+Oral only	Ocular & Oral only (probable clinical)
044	IIb, IIIa (1)	IIc, IIIa (1)	YES	absent	0	N/A no oral MMP	1:Ocular only	Ocular only (probable clinical)
045	IId, IIId (1)	IId, IIId (1)	YES	absent	0	N/A no oral MMP	1:Ocular only	Ocular only (definitive clinical)
048	0	0	N/A	N/A no ocular MMP	12	YES	2:Oral only	Oral only (probable clinical)
049	IIb, IIIb	IIb, IIIb	no	absent	0	no	3:Ocular+Oral only	Ocular & Oral only (definitive clinical)
050	0	0	N/A	N/A no ocular MMP	0	N/A no oral MMP	4:Other non-ocular	Skin ± other site (definitive clinical)
051	IIc, IIIb	IIa, IIId	YES	absent	22	YES	3:Ocular+Oral only	Ocular & Oral only (definitive clinical)
052	0	0	N/A	N/A no ocular MMP	6	YES	2:Oral only	Oral only (definitive clinical)
053	IId, IIIb	IIc, IIIa	YES	PRESENT	DS	DS / Unknown	1:Ocular only	Ocular only (probable clinical)
054	IId, IIId (5)	IId, IIId (2)	YES	absent	2	YES	5:Other Ocular/mix	Nasopharyngeal ± other site (definitive clinical)
055	IIc, IIIb (3)	IIc, III3 (3)	YES	absent	0	N/A no oral MMP	5:Other Ocular/mix	Skin ± other site (definitive clinical)
056	IId, IIb (2)	IId, IIb (2)	YES	PRESENT	0	N/A no oral MMP	1:Ocular only	Ocular only (definitive clinical)
057	0	0	N/A	N/A no ocular MMP	8	YES	2:Oral only	Oral only (probable clinical)
058	0	0	N/A	N/A no ocular MMP	16	YES	4:Other non-ocular	Nasopharyngeal ± other site (definitive clinical)
059	0	0	N/A	N/A no ocular MMP	24	YES	2:Oral only	Oral only (definitive clinical)
060	0	0	N/A	N/A no ocular MMP	0	no	2:Oral only	Oral only (definitive clinical)
062	IIa, IIIa	IIa, IIIa	no	absent	0	N/A no oral MMP	5:Other Ocular/mix	Nasopharyngeal ± other site (definitive clinical)
065	IIa, IIIa	IIa, IIIa	no	absent	0	N/A no oral MMP	1:Ocular only	Ocular only (definitive clinical)
066	IIa, IIIa	IIb, IIId	YES	PRESENT	6	YES	3:Ocular+Oral only	Ocular & Oral only (definitive clinical)
068	0	0	N/A	N/A no ocular MMP	0	no	2:Oral only	Oral only (definitive clinical)
069	I	0	N/A	N/A no ocular MMP	12	YES	2:Oral only	Oral only (definitive clinical)
071	IId, IIIa	IId, IIId	YES	PRESENT	10	YES	5:Other Ocular/mix	Skin ± other site (definitive clinical)
072	I	I	no	absent	DS	DS / Unknown	5:Other Ocular/mix	Skin ± other site (definitive clinical)
073	0	0	N/A	N/A no ocular MMP	20	YES	2:Oral only	Oral only (definitive clinical)
074	0	0	N/A	N/A no ocular MMP	12	YES	2:Oral only	Oral only (probable clinical)
076	0	0	N/A	N/A no ocular MMP	12	YES	2:Oral only	Oral only (definitive clinical)
077	0	0	N/A	N/A no ocular MMP	12	YES	4:Other non-ocular	Nasopharyngeal ± other site (definitive clinical)
078	0	0	N/A	N/A no ocular MMP	21	YES	2:Oral only	Oral only (definitive clinical)
079	0	0	N/A	N/A no ocular MMP	8	YES	4:Other non-ocular	Skin & nasopharyngeal ± other site (definitive clinical)
081	0	I	no	absent	31	YES	5:Other Ocular/mix	Skin & nasopharyngeal ± other site (definitive clinical)
082	0	0	N/A	N/A no ocular MMP	0	no	4:Other non-ocular	Skin ± other site (definitive clinical)
084	IIb, IIIb	IIc, IIIa	YES	absent	14	YES	3:Ocular+Oral only	Ocular & Oral only (definitive clinical)
096	IIIb (1)	IIIa (1)	no	absent	0	no	5:Other Ocular/mix	Nasopharyngeal ± other site (definitive clinical)
099	IIb, IIIb (4)	IId, IIId (6)	YES	PRESENT	0	no	5:Other Ocular/mix	Nasopharyngeal ± other site (definitive clinical)
102	IIc, IIIa (1)	IId, IIId (3)	YES	absent	DS	DS / Unknown	1:Ocular only	Ocular only (probable clinical)
107	IIb, IIIa (2)	IIb, IIIa (2)	no	absent	DS	DS / Unknown	1:Ocular only	Ocular only (probable clinical)

SEROLOGY RESULTS FOR CASES

Serology tests
1) ELISA BP180-NC16a MBL
2) ELISA BP230 MBL
3) IgA IIF Human SSS
4) IgG IIF Human SSS
5) Laminin 332 (Keratinocyte Footprint Assay)
NOTE: 0 = negative result; 1 = positive result

Patient study ID	1)	2)	3)	4)	5)
001	0	0	0	0	0
002	0	0	0	0	0
003	0	0	0	0	0
004	0	0	0	0	0
005	0	0	0	0	1
006	0	0	0	1	0
007	0	0	0	0	0
008	0	0	0	0	0
009	0	0	0	0	0
010	0	0	0	0	0
011	0	0	0	0	0
013	0	0	0	1	0
014	0	0	0	0	0
015	0	0	0	0	0
016	0	0	0	0	0
017	0	0	0	0	0
018	0	1	0	1	0
019	0	0	0	0	0
020	1	1	0	0	0
021	0	0	0	0	0
022	0	0	0	0	0
023	1	1	1	0	0
024	1	1	0	0	0
025	0	0	0	0	0
026	0	0	0	0	0
027	0	0	0	0	0
028	0	0	0	0	0
029	0	0	1	0	0
030	0	0	0	0	0
031	1	0	0	0	0
032	1	1	0	0	0
034	0	0	0	0	0
035	1	0	0	0	0
036	0	0	0	0	0
037	0	0	0	0	0
038	0	1	0	1	0
039	0	1	0	0	0
040	0	0	0	0	0
041	0	0	0	0	0
042	0	0	0	0	1
043	0	0	0	0	0
044	0	0	0	0	0
045	0	0	0	0	0
048	0	0	1	0	0
049	0	0	0	0	0
050	0	0	0	0	0
051	0	0	1	1	0
052	1	1	0	0	0
053	0	0	0	0	0
054	0	0	0	0	0
055	0	0	0	0	0
056	0	0	0	0	0
057	0	0	0	1	0
058	1	0	1	0	0
059	0	0	0	0	0
060	0	0	0	0	0
062	0	0	0	0	0
065	0	0	0	0	0
066	0	0	0	0	0
068	0	0	0	0	0
069	1	0	0	1	0
071	1	0	0	0	0
072	0	0	0	0	0
073	0	0	0	0	1
074	1	0	0	0	0
076	1	1	0	0	0
077	0	0	0	0	0
078	0	0	0	0	0
079	1	1	0	0	0
081	1	0	0	1	0
082	0	0	0	1	0
084	0	0	0	0	0
096	1	0	0	0	0
099	0	0	0	0	0
102	1	0	0	0	0
107	0	0	0	0	0

CONTROL DEMOGRAPHICS / **SEROLOGY RESULTS FOR CONTROLS**

Serology tests
1) ELISA BP180-NC16a MBL
2) ELISA BP230 MBL
3) IgA IIF Human SSS
4) IgG IIF Human SSS
5) Laminin 332 (Keratinocyte Footprint Assay)
NOTE: 0 = negative result; 1 = positive result

CONTROL ID	Race,	Gender	Age	Source	Systemic disease	Eye Disease	1)	2)	3)	4)	5)
CO1PW	white	M	71	Patient	Nil	Cataract	0	0	0	0	0
CO2DB	white	F	47	Staff	Nil	Squint	0	0	0	0	0
CO3HB	white	M	68	Patient	Nil	Cataract	0	0	0	0	0
CO4JG	white	F	54	Staff	Nil	Nil	0	0	0	0	0
CO5DA	white	M	64	Patient	Nil	Cataract	0	0	0	0	0
CO6LH	white	F	58	Patient	Nil	Nil	0	0	0	0	0
CO7NP	white	M	85	Patient	Nil	Cataract	0	0	0	0	0
CO8KW	white	M	47	Staff	Nil	Nil	0	0	0	0	0
CO9CP	white	F	58	Patient	Nil	Retinal detachment	0	0	0	0	0
CO10JD	white	M	60	Staff	Nil	Nil	0	0	0	0	0
CO11EK	white	F	51	Staff	Nil	Nil	0	0	0	0	0
CO12SL	white	F	60	Staff	Nil	Nil	0	0	0	0	0
CO13IM	white	F	59	Staff	Nil	Nil	0	0	0	0	0
CO14TS	white	F	63	Staff	Nil	Nil	0	0	0	0	0
CO15AD	white	F	58	Staff	Nil	Nil	0	0	0	0	0
CO16JA	white	F	75	Patient	Nil	Fuch's corneal dystrophy	0	0	0	0	0
CO17JD	white	F	69	Staff	Nil	Nil	0	0	0	0	0
CO18RC	white	M	74	Patient	Nil	Squint	0	0	0	0	0
CO19BC	white	M	61	Staff	Nil	Nil	0	0	0	0	0
CO20SJ	white	F	73	Patitnt	Nil	Cataract	0	0	0	0	0
CO21SM	asian	M	59	Patient	Nil	Keratoconus	0	0	0	0	0
CO22NB	white	F	73	Patient	Nil	Cataract, squint, basal cell carcinoma	0	0	0	0	0
CO23JP	white	F	69	Patient	Nil	Catarat, squint, lid surgery	0	0	0	0	0
CO24JD	white	F	61	Staff	Nil	Nil	0	0	0	0	0
CO25PD	white	M	51	Patiet	Nil	Squint	0	0	0	0	0
CO26RP	white	M	61	Patient	Nil	Cataract	0	1	0	0	0
CO27GS	white	M	86	Patient	Nil	Cataract	0	0	0	0	0
CO28SH	White	F	54	Staff	Nil	Nil	0	0	0	0	0
CO29JM	White	M	39	Staff	Nil	Nil	0	0	0	0	0
CO30HK	White	F	57	Patient	Nil	Cataract	0	0	0	0	0
CO31GF	White	M	57	Patient	Nil	Squint	0	0	0	0	0
CO32PK	White	M	83	Patient	Hypertension	Cataract	0	0	0	0	0
CO33DC	White	M	41	Staff	Nil	Nil	0	0	0	0	0
CO34RL	White	M	63	Staff	Nil	Nil	0	0	0	0	0
CO35RM	White	M	73	Patient	Nil	Glaucoma, lid surgery, maculopathy	1	0	0	0	0
CO36MM	White	F	55	Patient	Nil	Squint	0	0	0	0	0
CO37SH	White	M	54		Nil	Blepharoplasty	0	0	0	0	0
CO38GT	White	M	48	Staff	Nil	Nil	0	0	0	0	0
CO39MA	White	F	70	Patient	Nil	Herpes simplex keratitis	0	0	0	0	0
CO40PC	white	F	72	Patient	Nil	Fuch's cyclitis	0	0	0	0	0
CO41JL	white	F	67	Patient	Nil	Fuch's corneal dystrophy	0	0	0	0	0
CO42JS	white	M	71	Patient	Nil	Fuch's corneal dystrophy	0	0	0	0	0
CO43AP	white	F	81	Patient	Nil	Fuch's corneal dystrophy	0	0	0	0	0
CO44JU	asian	M	44	Staff	Nil	Nil	0	0	0	0	0
CO45KJ	african	F	18	Patient	Nil	Cataract	1	0	0	0	0

* Central Corneal conditions in either eye: 3 = central vessels, 5 = central scarring, 6 = central ulceration, 8 = central conjunctivalisation
** Current Systemic immunosuppression used: None 0, cyclophosphamide 1, mycophenolate 2, azathioprine 3, dapsone 4, methotrexate 5, Prednisolone 6, Rituximab 7, Ciclosporin 8, Other 9
*** Classification by main site of involvement uses rules outlined in text box below

Rules for the classification of MMP site of involvement using screening & history data		
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 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.
Skin ± other site	**Definitive clinical**	Screen positive or History positive or both
	Absent	Screen negative & History negative
	DS/Uncertain	Declined screening / not screened for the site
Nasopharyngeal ± other site	As for skin above	As for skin above
Genital ± other site	As for skin above	As for skin above

Serology test numbers	Antigens and substrates
1)	ELISA BP180-NC16a MBL
2)	ELISA BP230 MBL *
3)	BIOCHIP BP180-NC16a
4)	IgA IIF Human SSS
5)	IgG IIF Human SSS
6)	Laminin 332 (Keratinocyte Footprint Assay)

Supplemental Table (online only)

Supplementary expanded Table 2.

Test n#	Antigens and substrates	Test methodology, antibody specificity, supplier cut off level†	Positive Reactions n (%)			Sensitivity & Specificity			Positive Reactions n (%)		p-values (exact 2-sided)			MMP Phenotypes with Poitive Reactions n (%)				p-values (exact 2-sided) all *cf* Controls				Positive Reactions n (%)	
			MMP Cases (total n=76)	Controls (total n=45)	Exact p-value	Sn%	Sp%	Youden's Index	DIF Negative (total n=24)	DIF Positive (total n=49)	DIF-ve *cf* Ctrl	DIF+ve *cf* Ctrl	DIF-ve *cf* DIF+ve	Ocular only (n=18)	Oral only (n=14)	Ocular + Oral only (n=15)	Non-Ocular (n=20)	Ocular only	Oral only	Ocular + Oral only	Non-Ocular	DIF Positive* Non-Ocular MMP (n=19)	p-value *cf Controls*
		Enzyme-linked immunosorbent assays (ELISA)																					
1)	ELISA BP180-NC16a	Medical & Biological Laboratories (MBL) International®.	16 (21.05)	2 (4.44)	**0.016**	21.05	95.56	16.61	4 (16.67)	12 (24.49)	0.173	**0.008**	0.555	1 (5.56)	5 (35.71)	1 (6.67)	8 (40.00)	>0.999	**0.006**	>0.999	**0.001**	8 (42.11)	**0.001**
2)	ELISA BP230 MBL	Cut-off <9 U/ml	10 (13.16)	1 (2.22)	0.052	13.16	97.78	10.94	4 (16.67)	6 (12.24)	**0.046**	0.113	0.720	1 (5.56)	3 (21.43)	1 (6.67)	4 (20.00)	0.493	**0.038**	0.441	**0.028**	4 (21.05)	**0.024**
		Indirect immunofluorescence																					
3)	IgA IIF SSS	Human 1 molar salt split skin to detect IgG and IgA antibodies	5 (6.58)	0 (0.00)	0.156	6.58	100	6.58	1 (4.17)	4 (8.16)	0.348	0.118	>0.999	0 (0.00)	1 (7.14)	1 (6.67)	2 (10.00)	---	0.237	0.250	0.091	2 (10.53)	0.085
4)	IgG IIF SSS		9 (11.84)	0 (0.00)	**0.026**	11.84	100	11.84	1 (4.17)	7 (14.29)	0.348	**0.013**	0.258	1 (5.56)	3 (21.43)	2 (13.33)	4 (20.00)	0.286	**0.011**	0.059	**0.007**	3 (15.79)	**0.023**
		Other																					
5)	KFA: Laminin 332	KFA (Groningen): keratiocytre footprint assay for lanimin 332 antibody detection	3 (3.95)	0 (0.00)	0.233	3.95	100	3.95	0 (0.00)	3 (6.12)	---	0.243	0.546	0 (0.00)	1 (7.14)	2 (13.33)	1 (5.00)	---	0.237	0.059	0.308	1 (5.26)	0.297
Combined Reactions																							
1) + 2)			19 (25.00)	3 (6.67)	**0.014**	25.00	93.33	18.33	5 (20.83)	14 (28.57)	0.116	**0.007**	0.577	2 (11.11)	6 (42.86)	1 (6.67)	9 (45.00)	0.618	**0.004**	>0.999	**0.001**	9 (47.37)	**<0.001**
3) + 4)			13 (17.11)	0 (0.00)	**0.002**	17.11	100	17.11	2 (8.33)	10 (20.41)	0.118	**0.001**	0.315	1 (5.56)	4 (28.57)	2 (13.33)	6 (30.00)	0.286	**0.002**	0.059	**<0.001**	5 (26.32)	**0.002**
1) + 3) + 4)			25 (32.89)	2 (4.44)	**<0.001**	32.89	95.56	28.45	5 (20.83)	19 (38.78)	**0.045**	**<0.001**	0.185	2 (11.11)	8 (57.14)	3 (20.00)	12 (60.00)	0.571	**<0.001**	0.094	**<0.001**	11 (57.89)	**<0.001**
1) + 2) + 3) + 4)			26 (34.21)	3 (6.67)	**<0.001**	34.21	93.33	27.54	6 (25.00)	19 (38.78)	0.056	**<0.001**	0.300	3 (16.67)	8 (57.14)	3 (20.00)	12 (60.00)	0.341	**<0.001**	0.159	**<0.001**	11 (57.89)	**<0.001**
1) + 3) + 4) + 5)			28 (36.84)	2 (4.44)	**<0.001**	36.84	95.56	32.40	5 (20.83)	22 (44.90)	**0.045**	**<0.001**	0.070	2 (11.11)	9 (64.29)	5 (33.33)	13 (65.00)	0.571	**<0.001**	**0.008**	**<0.001**	12 (63.16)	**<0.001**
2) + 3) + 4) + 5)			23 (30.26)	1 (2.22)	**<0.001**	30.26	97.78	28.04	5 (20.83)	17 (34.69)	**0.017**	**<0.001**	0.284	2 (11.11)	7 (50.00)	5 (33.33)	10 (50.00)	0.194	**<0.001**	**0.003**	**<0.001**	9 (47.37)	**<0.001**
1) + 2) + 3) + 4) + 5)			29 (38.16)	3 (6.67)	**<0.001**	38.16	93.33	31.49	6 (25.00)	22 (44.90)	0.056	**<0.001**	0.128	3 (16.67)	9 (64.29)	5 (33.33)	13 (65.00)	0.341	**<0.001**	**0.019**	**<0.001**	12 (63.16)	**<0.001**

Test numbering is used in Figures 1-3.
† Commercially available tests were carried out according to the manufacturer's instructions.
* in the 20 non-ocular MMP cases 19 were DIF positive and 1 had no DIF result.

Supplementary Table 3 Proportions of patients with positive serology with and without active inflammation and/or systemic immunosuppression. Analysis for 74 patients having MMP with oral and/or ocular involvement, for whom the degree of inflammatory activity was graded using validated grading tools.[1,2] The raw data is available in Supp. Table 1 also available as an Excel Workbook at Mendeley Data https://data.mendeley.com/datasets/7pxbkx84r3/draft?a=02efd7af-8c11-4dc0-8be0-c45b93682bad

3A Positive serology tests in those with presence or absence of inflammation. Active inflammation is strongly associated with a positive serology result.

Active inflammation*	**Number**	**Positive serology****	**Fisher's exact test**
Absent	23	3 (13.04%)	p = 0.004
PRESENT	51	25 (49.02%)	
Total	**74**	**28 (37.84%)**	

3B Positive serology tests in those with and without systemic immunotherapy. Systemic immunotherapy is not associated with positive serology

Systemic immunotherapy†	**Number**	**Positive serology****	**Fisher's exact test**
Absent	31	10 (32.26%)	p = 0.471
PRESENT	43	18 (41.86%)	
Total	**74**	**28 (37.84%)**	

3C Interaction between systemic Immunotherapy, active Inflammation and positive serology tests. Findings suggest that association is with active inflammation rather that systemic immunosuppression

Systemic immunotherapy	**Active * Inflammation**	**Number**	**Positive ** serology**	**Fisher's exact test**
Absent	Absent	12	0	p = 0.004
Absent	PRESENT	19	10 (52.63%)	
PRESENT	Absent	11	3 (27.27%)	p = 0.309
PRESENT	PRESENT	32	15 (46.88%)	
Totals		**74**	**28 (37.84%)**	
Overall comparison				p = 0.006

* Active inflammation categorised as ocular inflammation score ≥5 and oral inflammation score ≥1 using the validated grading tools.[1,2]

** One or more of the 5 serology tests positive (ELISA BP180-NC16a MBL, ELISA BP230 MBL, IgA IIF SSS, IgG IIF SSS and anti-laminin 332 using the Keratinocyte footprint assay) see Table 1

† Systemic immunotherapy defined as any immunomodulatory drug given by intravenous or oral routes

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Supplementary Table 4
Summary of results of previous serology studies in MMP patients (Supplementary Table 3a) and normal controls (Table 3b) compared to those in the current study.

Summary of findings
To facilitate the discussion of our findings in relation to those of other studies we have reviewed 13 similar studies on serum reactivity to pemphigoid associated antigens in MMP in Supplementary Table 3a and of the prevalence of these antibodies in 3 control populations, having similar demographics and tests, to those in our study in Supplementary Table 3b. Our findings for the BP180 and BP230 ELISAs and Lam 332 are similar to those for other studies. On the other hand, our proportions of subjects having positive IgG IIF SSS are amongst the lowest reported; these range from 35-83.6% in other studies compared with ours of 11.84% overall cases, rising to 21.43% for pure oral MMP[9], similar to those ofCalabresi et al. For IgA IIF SSS our findings are comparable to those of other studies in which reported proportions vary from 0 in ocular disease to 10-11% in oral[9] (Calabresi et al) and predominantly oral MMP[7] (Carrozo et al.) as opposed to our findings of 6.58% overall rising to 10% in non-ocular MMP cases; however, for multisite MMP the proportions positive from some laboratories have been as high as 62%.6,3, (Setterfield J et al. 2001, Setterfield J at al. 1998, Oyama et al. 2006) These differences in the proportions of routine tests that are positive probably relate to; differences in serum reactivity for MMP involving different sites with sites including ocular disease having lower reactivity; variations in laboratory techniques and variations in disease activity. Given the low sensitivity of serology tests in MMP and the false positive rate in controls for IIF SSS of 1-6%, and for the ELISA's of 2-6% (see Supplementary Table 3b) our study has shown that the finding of a positive result must be interpreted with caution before using this as confirmation of a diagnosis of MMP. As in our study, future studies of serum pemphigoid antibody detection in MMP should use an appropriately matched control population to validate the interpretation of results.

Table 4a
Summary of results of previous serology studies in MMP compared with current study.

PREVIOUS STUDIES						**CURRENT STUDY**	
AUTHOR Date	**MMP SITES INVOLVED** *(Number of cases)* *Description* Ocular [involvement][1] n (%)	**DIF+** Number (%) *Description*	**TEST METHOD**	**RESULTS** Number (%)	**CONTROLS** Number (%) *Description*	**CASES** Number (%) Results for all 76 cases unless otherwise stated Cases with ocular involvement 56/76 (73.68%) Ocular only cases 18/76 (23.7%) 49/76 cases were DIF+	**CONTROLS** Age, sex race matched (n 45)
Balding S 1996(Balding, Prost et al. 1996)	**Multiple sites** (23) Ocular 8/23 (34.8%)	18/18	IgG IIF SSS	11/23 (47.8%)	None	**DIF+ cases only** 7/49 (14.29%)	NA
			Lam 332	0/18 [IB]		3/76 (3.95%) [KFA]	
Murakami H 1998(Murakami, Nishioka et al. 1998)	**Ocular and oral only** (50) *Ocular unreported*	*Number uncertain*	IgA IIF SSS	22/50 (44%)	None	**Ocular and oral only** 2/47 (4.25%)	NA
			IgG IIF SSS	19/50 (38%)	None	6/47 (12.7%)	
Setterfield J 1998(Setterfield, Shirlaw et al. 1998)	**Multiple sites** (67) *Ocular 62/67 (91%)*	64 (95.5%)	IgA SSS	41/67 (61.2%)	Controls but unreported	**DIF+ cases only** 4/49 (8.16%)	NA
			IgG SSS	56/67 (83.6%)		7/49 (14.29%)	
Leverkus M 1999(Leverkus, Schmidt et al. 1999)	**Multiple sites** (16) *All with scarring* Ocular 9/16 (56.2%)	16 (100%)	ELISA BP180-NC16a	2/14 (14.3%)		**DIF+ cases only** 12/49 (24.49%)	NA
			IgG IIF SSS	9/16 (56%)		20/49 (34.7%)	
			Lam 332	5/16 (31.3%) [IB]		3/76 (3.95%) [KFA]	

Schmidt E 2001(Schmidt, Skrobek et al. 2001)	**Multiple sites** (26) *All with scarring* Ocular 19/26 (73.1%)	26 (100%)	IgA IIF SSS	6/26 (23.1%)	20 unreported	**DIF+ cases only** 4/49 (8.16%)	NA
			IgG IIF SSS	12/26 (46.2%)		7/49 (14.29%)	
			Lam 332 IB	7/26 (26.9%)	20 but results unreported	3/49 (6.12%) [KFA]	
Setterfield J 2001(Setterfield, Theron et al. 2001)	**Multiple sites** (131) Ocular 100/131 (76.3%)	111 (84.7%)	IgA IIF Hum SSS	70/131 (55.1%)	None	**DIF+ cases only** 4/49 (8.16%)	NA
			IgG IIF Hum SSS	92/131 (72.4%)	None	7/49 (14.29%)	
Carrozzo M 2004(Carrozzo, Cozzani et al. 2004)	**Predominantly oral (28)** 19/28 oral only 9/28 oral & other sites Ocular 4/28 (14%)	27 (96.4%)	IgA IIF Hum SSS	3/28 (10.7%)	*20 healthy & 20 with lichen planus* 0/40	**Oral and non-ocular** 3/34 (8.8%)	0/45
			IgG IIF Hum SSS	12/28 (42.9%)	0/40	7/34 (20.5%)	0/45
Oyama N 2006(Oyama, Setterfield et al. 2006)	**Multiple sites** (124) Ocular 96/124 (77.4%)	101 (81.4%)	IgA IIF SSS	77/124 (62%)	None	**DIF+ cases only** 4/49 (8.16%)	0/45
			IgG IIF SSS	102/124 (82%)		7/49 (14.29%)	
Calabresi V 2007 (Calabresi, Carrozzo et al. 2007)	**Oral only (**20) *Untreated*	20 (100%)	**Oral only** IgA IIF SSS	**Oral only** 2/20 (10%)	None	**Oral only** 1/14 (7.14%)	0/45
			IgG IIF SSS	7/20 (35%)		3/14 (21.43%)	0/45
Jonkman M 2009(Jonkman, Groot et al. 2009)	**Ocular only** (11)	5 (45.5%)	**Ocular only** IgA IIF SSS	**Ocular only** 0/9	None	**Ocular only** 0/18	NA 0/45
			IgG IIF SSS	4/10 (40%)		1/18 (5.56%)	0/45
Bernard P 2013(Bernard, Antonicelli et al. 2013)	**Multiple sites** (154) Ocular 68/154 (44.2%)	154 (100%)	ELISA BP180-NC16a	60/154 (38.9%)	None	16/76 (21.05%)	2/45 (4.44%)
			ELISA BP 230	16/154 (10.4%)		10/76 (13.16%)	1/45 (2.22%)
Hayakawa T 2014(Hayakawa, Furumura et al. 2014)	**Non-ocular** (30) *Predominantly oral additional non-ocular sites in 5/30*	30 (100%)	**Non-ocular** ELISA BP180-NC16a	**Non-ocular** 9/30 (30.0%)	None	**Non-ocular** 13/34 (38.23%)	NA
			ELISA BP230	0/30		7/34 (20.5%)	
			IgA IIF Hum SSS	8/30 (26.7%)		3/34 (8.82%)	
			IgG IIF Hum SSS	18/30 (60.0%)		7/34 (20.5%)	
			Lam 332	7/30 (23.3%) [IB]		2/34 (5.88%) [KFA]	
Cozzani E 2016(Cozzani, Fontana et al. 2016)	**Multiple sites** (78) Ocular only 10/78 (12.8%) Ocular 25/78 (32%)	78 (100%)	**DIF + cases only** ELISA BP180-NC16a	**DIF + cases only** 6/78 (33%)		**DIF + cases only** 12/49 (24.49%)	0/45
			ELISA BP230	9/78 (11.5%)		6/49 (12.24%)	
			Lam 332	9/78 (11.5%) [IB]	10 *controls for Lam 332 IB only* 0/10	3/49 (6.12%) [KFA]	

Footnotes are common to Supplementary Tables 3a and 3b and are found after Table 3b

Supplementary Table 4b

Summary of results of previous studies on the prevalence of circulating pemphigoid antibodies in control populations compared to those in the current study.

PREVIOUS STUDIES					**CURRENT STUDY (n=45)**
Author	**Number and matching criteria**	**DIF results**	**Antibody detection method**	**Results**	**Results**
Desai N 2008(Desai, Allen et al. 2008)	61 healthy, mainly female, 50-70 yrs	Not done	ELISA BP180-NC16a	0/20	2/45 (4.44%)
			IgA IIF Hum SSS	0/61	0/45
			IgG IIF Hum SSS	3/61 (4.9%)	0/45
			Non-comparable tests: BP180 immunoblot 35/61 (57%) positive & BP 230 immunoblot 6/61 (9%) positive	**Total positive (3 tests) 3/61 (4.9%)** note not all tests done in every patient	**Total positive (3 tests) 2/45 (4.4%)**
Hachisuka H 1996(Hachisuka, Kurose et al. 1996)	32 healthy older (60-90 yrs) Note: 28 healthy younger (20-30 yrs) controls had negative results	6/6 negative	IgG IIF Hum SSS	1/32 (3%) **Total 3%**	0/45 **Total 0**
Wieland CM 2010(Wieland, Comfere et al. 2010)	337 age & sex stratified (20-90 yrs. 20 of each sex per decade) controls from a registry having celiac disease, pemphigus and pemphigoid excluded	Not done	ELISA BP180-NC16a (MBL)	14/337 (4.15%)	2/45 (4.44%)
			ELISA BP230 (MBL)	14/337 (4.15%)	1/45 (2.2%)
				Total positive (2 tests) 25/337 (7.4%)	**Total (2 tests) 2/45 (4.4%)**

van Beek N 2014(van Beek, Dohse et al. 2014)	93 patients with non-inflammatory skin disease aged ≥70 (mean 78)	Not done	BP180 NC16A ELISA (MBL)	3.25% approx.	2/45 (4.44%)
			BP230 ELISA (MBL)	6.25% approx.	1/45 (2.22%)
			IgG IIF Hum SSS	1% approx	0/45
			Lam 332	None	0/45
			Non comparable tests percentages positive in brackets (numbers not given): IgG MO Es (2%), BP180 NC16A ELISA (Euroimmun) (6.5%), BP 230 ELISA (Euroimmun) (7.75%)	**Total positive:** uncertain	**Total positive**: non comparable
van Beek N 2014(van Beek, Dohse et al. 2014)	50 blood donors mean age 41	Not done	BP180 NC16A ELISA (MBL)	None	2/45 (4.44%)
			BP230 ELISA (MBL)	7. 6% approx.	1/45 (2.22%)
			IgG IIF Hum SSS	2. 2% approx.	0/45
			Lam 332	None	0/45
			Non comparable tests percentages positive in brackets (numbers not given): IgG MO Es (2%), BP180 NC16A ELISA (Euroimmun) (2%), BP 230 ELISA (Euroimmun) (none positive). Immunoblots IgG to LAD1, BP180, BP230	**Total positive:** non comparable	**Total positive:** non comparable

Footnotes

[1]Ocular [involvement] reported as Ocular for any case with ocular involvement.

DIF+ = positive direct immunofluorescence**;** IIF Hum SSS = Indirect immunofluorescence using human 1 mol/l salt split skin**;** IIF SSS = Indirect immunofluorescence on salt split skin unspecified species (probably human)
IB = Immunoblotting; IIF = Indirect Immunofluorescence; MO Es = monkey esophagus; NA= not applicable; approx. = approximately

Rules for comparing previous studies with data from the current study

Composition of cases

Studies with multiple sites of involvement including predominantly (>95%) DIF+ cases are compared with our DIF+ cases (n=49/73 [67.1%]).
Studies with multiple sites of involvement included (between 80-85% of DIF+ cases), or no DIF results, are compared with all of our 76 cases (of which 67.1% DIF+).
Studies with oral only and ocular only cases are compared with our oral only and ocular only cases.
Studies with non-ocular cases are compared with our non-ocular cases.

Tests compared

Only tests using the same methodology and substrate were compared unless otherwise stated.
For reported BP180-NC16A ELISA results comparisons are with our MBL BP180-NC16A ELISA unless otherwise stated
Tests that were not the same in terms of substrate or methodology are not reported

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