ORIGINAL ARTICLE

Multiple and repeated sampling increases the sensitivity of direct immunofluorescence testing for the diagnosis of mucous membrane pemphigoid

Iakov Shimanovich, MD, Julia Marie Nitz, and Detlef Zillikens, MD
Lübeck, Germany

Background: Mucous membrane pemphigoid (MMP) is an autoimmune disease characterized by the predominant blistering of mucosal surfaces and the linear deposition of complement, IgG, or IgA along the basement membrane detected by direct immunofluorescence (DIF) test.

Objective: To assess the impact of multiple and repeated DIF sampling on establishing the diagnosis of MMP.

Methods: We reviewed the results of DIF studies in 136 nonlesional biopsies from 78 patients who were immunologically confirmed to have MMP.

Results: Thirty-six of 52 patients (69%) who underwent only 1 biopsy at the first workup were positive. In 13 cases, the initial single biopsy was negative, and later biopsies were positive. Twenty-two of 26 patients (85%) who underwent multiple biopsies at the initial workup showed ≥1 positive DIF test result. Simultaneously obtained biopsies yielded discordant positive and negative findings in 11 patients. Overall, 74 of 78 patients (95%) had ≥1 positive result by DIF test. In the remaining 4 cases, the diagnosis was confirmed by the detection of circulating autoantibodies against BP180.

Limitations: This is a retrospective, single-center study.

Conclusion: Our data demonstrate that multiple and repeated biopsies increase the sensitivity of the DIF test for MMP diagnosis. Negative DIF test findings in cases clinically suggestive of MMP should prompt repeat biopsies. (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2017.05.016.)

Key words: biopsy; diagnosis; immunofluorescence; mucous membrane pemphigoid; sensitivity.

Mucous membrane pemphigoid (MMP) is an autoimmune, subepidermal blistering disease that predominantly involves mucosal surfaces. Disease is mediated by IgG and IgA autoantibodies against the mucosal and cutaneous basement membrane zone. The circulating antibodies are most commonly directed against bullous pemphigoid (BP) antigen 180 (BP180, type XVII collagen), laminin 332 (laminin 5, epiligrin), type VII collagen, or β4 integrin. They are usually present at a low titer and are difficult to detect. Therefore,

Abbreviations used:

BP:	bullous pemphigoid
DIF:	direct immunofluorescence
ELISA:	enzyme-linked immunosorbent antibody assay
MMP:	mucous membrane pemphigoid

From the Department of Dermatology, University of Lübeck.
Funding sources: None.
Conflicts of interest: None declared.
Previously presented: The study was presented at the 75th American Academy of Dermatology annual meeting in Orlando, Florida, March 3-7, 2017.
Accepted for publication May 14, 2017.

Reprint requests: Iakov Shimanovich, MD, Department of Dermatology, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: iakov.shimanovich@uksh.de.
Published online July 1, 2017.
0190-9622/$36.00

http://dx.doi.org/10.1016/j.jaad.2017.05.016

direct immunofluorescence (DIF) testing of perilesional tissue biopsies demonstrating linear deposits of complement, IgG, or IgA along the basement membrane is deemed to be the most sensitive method and a prerequisite for diagnosing MMP.[1,2]

Over the decades various studies have tried to assess the value of the DIF test for diagnosing MMP, usually reporting sensitivity rates of approximately 70%-80%.[3-7] However, in all of these studies the diagnosis of MMP for DIF-negative cases was established only on clinical and histopathologic grounds, which is not in line with the diagnostic criteria set out by the consensus statement on MMP.[2] It also has been noted that in some patients with ocular MMP the initial DIF test might be negative, and later biopsies might still yield positive diagnostic findings.[8] However, the impact of multiple sampling on the sensitivity of the DIF test for the diagnosis of MMP has not been studied in detail. We retrospectively evaluated 78 patients with the clinical phenotype of MMP; in all, the diagnosis was confirmed by characteristic DIF test findings, by detection of circulating autoantibodies against specific basement membrane proteins, or both. These data provide insights into the sensitivity of the DIF test in genuine MMP and demonstrate the importance of repeated and multiple sampling for establishing this diagnosis.

CAPSULE SUMMARY

- Direct immunofluorescence is essential for diagnosing mucous membrane pemphigoid.
- A single direct immunofluorescence study may yield false-negative results in a substantial number of patients.
- Multiple and repeated biopsies increase the sensitivity of direct immunofluorescence and should be the standard of care for patients with suspected mucous membrane pemphigoid.

METHODS

The results of all DIF, immunoserology, and histopathology studies performed from 2005 through 2015 in the Autoimmune Laboratory of the Department of Dermatology at the University of Lübeck related to the diagnosis of MMP were reviewed. Subsequently, patient histories were studied, and only cases with the clinical phenotype typical of MMP were included. Biopsies that contained areas of subepithelial splitting taken for DIF testing were excluded from the analysis.

For DIF tests, tissue specimens were snap-frozen at −20°C, and 6-μm cryosections were stained with fluorescein isothiocyanate-tagged antibodies against human complement C3 (BioLogo, Kronshagen, Germany), IgG, and IgA (both antibodies; Bio-Rad Laboratories, Marne-la-Coquette, France) as described previously.[9]

Previously published protocols were followed for indirect immunofluorescence of patient sera on 1 M NaCl-split normal human skin (IgG and IgA); enzyme-linked immunosorbent antibody assay (ELISA) with recombinant BP180 NC16A (IgG); ELISA with recombinant BP230 C3 (IgG); immunoblotting of recombinant BP180 4575; immunoblotting of concentrated conditioned medium, cellular extract, and extracellular matrix of cultured HaCaT keratinocytes (all IgG and IgA); and immunoblotting of human dermal extracts (IgG).[9-14] The values for sensitivity were calculated with the standard formula: sensitivity = true-positives/(true-positives + false-negatives). This study was approved by the Ethics Committee of the University of Lübeck.

RESULTS

A total of 136 nonlesional biopsies from 78 patients with MMP (34 men and 44 women aged 23-93 [median 65] years) were examined by DIF test (Supplemental Table I; available at http://www.jaad.org). At the initial workup, 52 of 78 patients had only 1 biopsy taken for DIF staining and the remaining 26 patients had ≥2 biopsies taken (Fig 1).

Thirty-six of 52 patients who underwent only 1 biopsy at the first diagnostic workup had a positive DIF test result (sensitivity 69%). In 13 of 52 cases, the initial single biopsy was negative, but ≥1 biopsies taken at later workups were positive. In the remaining 3 patients, repeat biopsies were not performed because the diagnosis of MMP was confirmed by the detection of circulating autoantibodies against BP180 (Fig 1).

Twenty-two of 26 patients who had ≥2 simultaneous biopsies at the initial workup showed ≥1 positive DIF test result (sensitivity 85%). In the remaining 4 patients, multiple initial biopsies were completely negative. Therefore, DIF staining was repeated and found to be positive in 3 cases. In 1 patient, DIF staining was negative again, but circulating autoantibodies against BP180 were present (Fig 1).

Overall (ie, at the initial and later visits), multiple simultaneous biopsies were obtained in 32 patients. In 21 of these patients, simultaneous biopsies showed concordant (ie, similar) findings (in 17 cases DIF test results were concordantly positive and in 4