In vitro diagnostics for the medical dermatologist. Part I: Autoimmune tests

Kelsey B. Nusbaum, BS,[a] Abraham M. Korman, MD,[b] Kelly Tyler, MD,[b] Jessica Kaffenberger, MD,[b] John Trinidad, MD,[b] and Benjamin H. Kaffenberger, MD, MS[b]
Cincinnati and Columbus, Ohio

Learning objectives
After completing this learning activity, participants should be able to discuss the strengths and weaknesses of various laboratory tests used to test for immunologic causes of skin disease; become familiar with new laboratory tests for detecting and diagnosing immunologic causes of skin disease; and recognize potential sources for discordant laboratory testing.

Disclosures
Editors
The editors involved with this CME activity and all content validation/peer reviewers of the journal-based CME activity have reported no relevant financial relationships with commercial interest(s).

Authors
The authors involved with this journal-based CME activity have reported no relevant financial relationships with commercial interest(s).

Planners
The planners involved with this journal-based CME activity have reported no relevant financial relationships with commercial interest(s). The editorial and education staff involved with this journal-based CME activity have reported no relevant financial relationships with commercial interest(s).

Despite the expansion of available in vitro laboratory tests at a rate far exceeding that of dermatologic pharmaceuticals, the existing literature is dominated by discussion of the latter. With the advent of numerous new tests, it can be difficult for practicing dermatologists to stay up-to-date on the available options, methodologies, and recommendations for when to order one test over another. Understanding the inherent strengths and weaknesses of these options is necessary to inform appropriate ordering and proper interpretation of the results. The first article in this continuing medical education series summarizes information on methodology, test characteristics, and limitations of several in vitro laboratory tests used for the work up of undifferentiated patients suspected of having dermatologic autoimmune diseases and it provides a general guide to ordering these tests. (J Am Acad Dermatol 2021;85:287-98.)

Key words: antineutrophil cytoplasmic antibody; antinuclear antibody; autoimmune; autoimmune blistering diseases; bullous pemphigoid; dermatitis herpetiformis; dermatomyositis; diagnostic testing; epidermolysis bullosa acquisita; inflammatory bowel disease; medical dermatology; pemphigus.

From the University of Cincinnati College of Medicine[a]; and Division of Dermatology, Department of Internal Medicine, The Ohio State University.[b]
Funding Sources: None.
Presented at the American Academy of Dermatology, grand rounds, and state society meetings by Dr Kaffenberger.
IRB approval status: Not applicable.
Accepted for publication February 24, 2021.
Reprints not available from the authors.
Correspondence to: Benjamin H. Kaffenberger, MD, MS, The Ohio State University Department of Internal Medicine, 1328 Dublin Road, Suite 100, Columbus OH 43212. E-mail: Benjamin.Kaffenberger@osumc.edu.
0190-9622/$36.00

https://doi.org/10.1016/j.jaad.2021.02.090

Date of release: August 2021.
Expiration date: August 2024.

Scanning this QR code will direct you to the CME quiz in the American Academy of Dermatology's (AAD) online learning center where after taking the quiz and successfully passing it, you may claim *1 AMA PRA Category 1 credit*. NOTE: You must have an AAD account and be signed in on your device in order to be directed to the CME quiz. If you do not have an AAD account, you will need to create one. To create an AAD account: go to the AAD's website: www.aad.org.

Abbreviations used:

ANA:	antinuclear antibody
ANCA:	antineutrophil cytoplasmic antibody
DIF:	direct immunofluorescence
dsDNA:	double-stranded DNA
ELISA:	enzyme-linked immunosorbent assay
IBD:	inflammatory bowel disease
IIF:	indirect immunofluorescence
MMP:	mucous membrane pemphigoid
MPO:	myeloperoxidase
MSA:	myositis-specific antibodies
PR3:	proteinase 3
SLE:	systemic lupus erythematosus

INTRODUCTION

In vitro diagnostics have increased at a rate far greater than new dermatologic pharmaceuticals. This article discusses updates on autoimmune diagnostics and their interpretation relevant to the medical dermatologist, including laboratory testing for antinuclear antibodies and antineutrophil cytoplasmic antibodies, as well as tests associated with dermatomyositis, autoimmune blistering disorders, and inflammatory bowel disease (IBD).

ANTINUCLEAR ANTIBODIES TESTING

Scenario: A 35-year-old woman presents with a history suspicious for systemic lupus erythematosus (SLE). Multiplex antinuclear antibodies (ANA) testing is ordered and the results are negative. Does this exclude the possibility of SLE (Fig 1)?

Key points

- Immunofluorescence remains the gold standard test for detection of antinuclear antibodies.
- If a patient clinically demonstrates features of SLE but tests negative for ANA with multiplex, the test should be reordered by immunofluorescence.
- Scenario interpretation: A negative ANA by multiplex is less sensitive than immunofluorescence and a negative result does not exclude SLE. Traditionally, indirect immunofluorescence has been performed for the detection of ANA but multiplex immunoassays are an automated, labor-efficient means of testing for ANA.

Indirect immunofluorescence

Indirect immunofluorescence continues to be the preferred and most widely used technique for ANA testing. Patient serum is placed on a slide containing HEp-2 cells or tissue. If present, ANAs bind specific antigens on the slide. Fluorescein-labeled immunoglobulin (Ig)G is added and the slide is viewed under a fluorescence microscope to identify staining pattern and titer.[1] ANA fluorescent patterns are categorized in 3 major groups: nuclear, cytoplasmic, and mitotic.[2-4] Certain patterns are reported by laboratories given their ease of recognition and association with clinical disease (Table I).[4] Nuclear patterns of mandatory reporting include homogenous, speckled, centromere, discrete nuclear dots, and nucleolar. Cytoplasmic patterns include fibrillary, speckled, reticular, polar, rods, and rings.[2-4] Clinical relevance of mitotic patterns is less understood and not currently required to be reported. ANA titer is determined by performing serial dilutions, with higher titers more predictive of autoimmune disease.[5,6]

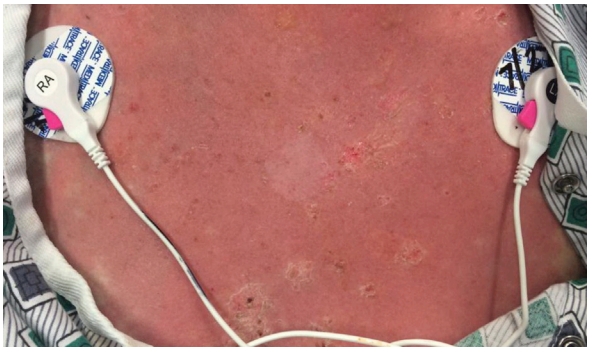

Fig 1. A young woman with photodistributed dusky erythema, dyspigmentation, and erosions over the sun-exposed surfaces of the face, chest, and arms, with fevers and cervical lymphadenopathy and new joint pain in her hands.

Indirect immunofluorescence is an extremely sensitive screening test with very few false negatives.[1,7,8] It detects a wide range of autoantibodies with broad screening potential, as HEp-2 cells contain up to 150 possible autoantigens.[9] Despite the high sensitivity of immunofluorescence, it is time consuming, often resulting in longer processing times. It is also labor intensive. The subjectivity of interpreting fluorescence intensity and staining pattern limits standardization and may lead to slight discrepancies between laboratories or repeated tests.[10] Additionally, positive ANA by immunofluorescence can be seen in up to 20%-30% of healthy individuals without apparent systemic autoimmune disease, highlighting the importance of correlating results with clinical presentation.[6,11] Use of a higher titer cutoff (e.g., 1:160) may reduce the incidence of false positives but at the expense of failing to detect individuals with disease.[6,11]

Multiplex immunoassay

Multiplex immunoassays are automated flow cytometry-based assays that allow simultaneous detection of multiple autoantibodies within a single specimen. In these assays, antigenic microbeads labeled with laser-reactive dyes are mixed with serum and a fluorochrome secondary antibody. As the microbead passes through the flow cell, it is

Table I. Clinical relevance of immunofluorescence staining patterns*

Staining pattern	Clinical relevance
Nuclear	
Homogenous	Observed in patients with SLE in association with anti-dsDNA antibodies; also present in chronic autoimmune hepatitis and juvenile idiopathic arthritis
Speckled	Lacks specificity for particular diagnosis; commonly observed in healthy individuals and wide range of autoimmune diseases
Centromere†	Strongly associated with limited cutaneous systemic sclerosis
Discrete nuclear dots	Multiple nuclear dot pattern is observed in a range of autoimmune diseases, while the few nuclear dot pattern has low predictive value for any disease
Nucleolar	Frequently observed in patients with systemic sclerosis and also seen in Sjögren syndrome
Cytoplasmic	
Fibrillary	Associated with autoimmune hepatitis, chronic HCV infection, and celiac disease
Speckled	Associated with SLE, inflammatory myopathy (including dermatomyositis), interstitial lung disease, and Raynaud's phenomenon
Reticular	Associated with primary biliary cholangitis, systemic sclerosis, and Sjögren syndrome
Polar	No strong clinical associations
Rods and rings	Observed in patients with HCV treated with interferon-α/ribavirin combination therapy

ANA, Antinuclear antibodies; *dsDNA*, double stranded DNA; *HCV*, hepatitis C virus; *SLE*, systemic lupus erythematosus.
*Clinical relevance of HEp-2 indirect immunofluorescence patterns based on latest International Consensus on ANA Pattern (ICAP) initiative.[4]
†Of the described patterns, only the centromere staining pattern is strongly associated with 1 specific disease phenotype.

Table II. Autoantibodies included on a commercially available multiplex panel and their association with autoimmune disease

Autoantibody	Clinical associations
dsDNA	Highly specific for SLE; serum levels correlate with disease activity, particularly of renal disease, and are predictive of renal flare; repeat testing may be useful for patient follow up
Sm	Highly specific for SLE and useful for identifying patients with negative anti-dsDNA and high clinical suspicion; associated with lupus nephritis
Chromatin	Highly specific for SLE and associated with lupus nephritis
Ribosomal P	Highly specific for SLE and associated with neuropsychiatric involvement
RNP	Diagnostic of mixed connective tissue disease with high titers; low titers may also be observed in SLE and systemic sclerosis
SS-A (Ro) and SS-B (La)	The main antibodies detected in Sjögren syndrome; also observed in SLE with associated skin and hematologic manifestations, subacute cutaneous lupus erythematosus, and neonatal lupus with increased risk of congenital heart block
Scl-70	Associated with diffuse systemic sclerosis and increased risk of pulmonary fibrosis
Centromere B	Associated with limited systemic sclerosis and increased risk of pulmonary arterial hypertension
Jo-1	Myositis-associated antibody often seen in anti-synthetase syndrome

dsDNA, Double-stranded DNA; *RNP*, ribonucleoprotein; *Scl*, scleroderma; *SLE*, systemic lupus erythematosus; *Sm*, Smith; *SS*, Sjögren syndrome.

interrogated by lasers to identify the coated antigen and relative amount of autoantibody bound.[12,13] Sensitivity is limited by using only the most common autoantibodies. Antibodies tested on 2 nationally available panels include double-stranded DNA (dsDNA), Smith, ribonucleoprotein, chromatin, Sjögren syndrome A and B, scleroderma-70, Jo-1, ribosomal P, and centromere B.[14,15] The clinical relevance of these autoantibodies is summarized in Table II.[6,16-18]

Multiplex immunoassays perform well in comparison to individual enzyme-linked immunosorbent assays (ELISAs) for each individual autoantibody.[12,19,20] However, sensitivity is only 58%-79% when compared to immunofluorescence, because the test does not exhaustively detect every possible autoantibody.[13,21-23] The cost is greater with multiplex compared to immunofluorescence due to the automatic reflexing needed to identify specific autoantibodies (Table III).

Table III. Summary of test characteristics and costs

Test	Sensitivity	Specificity	Gold standard test for comparison	2017 Medicare national limit
Antinuclear antibody immunofluorescence	>95%	57%-78%	ACR criteria for newly diagnosed systemic lupus erythematosus	$16.58 + 15.31 if positive to reflex to titer
Antinuclear antibody multiplex	58%-79%	99%	Immunofluorescence	$16.58 + 24.60 for each antibody reflexed
Immunofluorescence (c-ANCA)	65%-77%	97%-98%	ACR criteria newly diagnosed granulomatosis with polyangiitis (c-ANCA and PR3) and microscopic polyangiitis (p-ANCA and MPO)	$20.65 x (up to 3)
Immunofluorescence (p-ANCA)	85%-89%	81%-96%		
PR3 ELISA	77%-81%	98%-99%		$20.65 x (up to 3)
MPO ELISA	71%-88%	96%-99%		
BP180 ELISA	54%-95%	90%-99%	Direct immunofluorescence for bullous pemphigoid	$15.82 x (up to 2)
BP230 ELISA	48%-82%	65%-99%		
Desmoglein 1 ELISA	92%-98%	98%-100%	Direct immunofluorescence for pemphigus	
Desmoglein 3 ELISA	94%-100%	98%-100%		
Collagen 7 ELISA	86%-99%	98%-99%	Direct immunofluorescence for epidermolysis bullosa acquisita	
Anti-tTG ELISA	47%-95%	98%-100%	Direct immunofluorescence for dermatitis herpetiformis	
Anti-eTG ELISA	52%-100%	93%-100%		
IgG ASCA	41%-84%	86%-96%	Endoscopically confirmed inflammatory bowel disease	$16.81 x (up to 2)
IgA ASCA	34%-84%	84%-95%		
IBD combination panel*				$248.6 + unlisted molecular fee x 4
Crohn disease	89%	81%		
Ulcerative colitis	98%	84%		
All IBD	74%	90%		
Fecal calprotectin	93%	96%		$26.93

ACR, American College of Rheumatology; *ANCA*, antineutrophil cytoplasmic antibodies; *anti-eTG*, anti-epidermal transglutaminase; *anti-tTG*, anti-tissue transglutaminase; *ASCA*, anti-saccharomyces cerevisiae antibodies; *BP*, bullous pemphigoid; *ELISA*, enzyme-linked immunosorbent assay; *IBD*, inflammatory bowel disease; *MPO*, myeloperoxidase; *PR3*, proteinase 3.
*Specifically Prometheus.

Recommended approach to ordering

It is recommended that ANA is ordered when the history and physical examination elicit suspicion of systemic autoimmune disease.[4,11] Immunofluorescence remains the gold standard for ANA testing.[24] Laboratories should report the methodology of testing, given the high variability between sites.[24,25] Immunofluorescence should be performed if the patient is clinically considered to be at high risk for SLE but tests negative with multiplex. In the case of a positive dsDNA result on multiplex accompanied by negative immunofluorescence ANA, immunofluorescence on the more specific *Crithidia luciliae* substrate may help delineate a true result.[26]

It is not recommended that panels of ANA subserologies are ordered without a positive ANA and clinical suspicion of immune-mediated disease.[27] Repeated ANA by immunofluorescence has little clinical value in monitoring disease activity or predicting a flare, with the exception of dsDNA and antichromatin antibodies.[28] Complement studies can be considered as an adjunct to evaluate SLE activity, but low levels of C3 and C4 are also observed in other autoimmune conditions and infectious diseases.[29,30]

ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES TESTING

Scenario: A 20-year-old woman presents with rapidly progressive, cribriform ulceration of her right shin, weakness, and bloody diarrhea. Antineutrophil cytoplasmic antibody (ANCA) testing is performed as part of testing to evaluate for medium-vessel vasculitis. Although Proteinase 3 (PR3) and MPO autoantibodies return a negative result, indirect immunofluorescence returns a positive result for atypical p-ANCA. What is the most common association for an atypical p-ANCA (Fig 2)?

Key points

- Antigen-specific assays for PR3-ANCA and MPO-ANCA should be the primary screening method for ANCA.
- Scenario interpretation: Atypical p-ANCA is associated with multiple autoimmune diseases but most significantly ulcerative colitis.

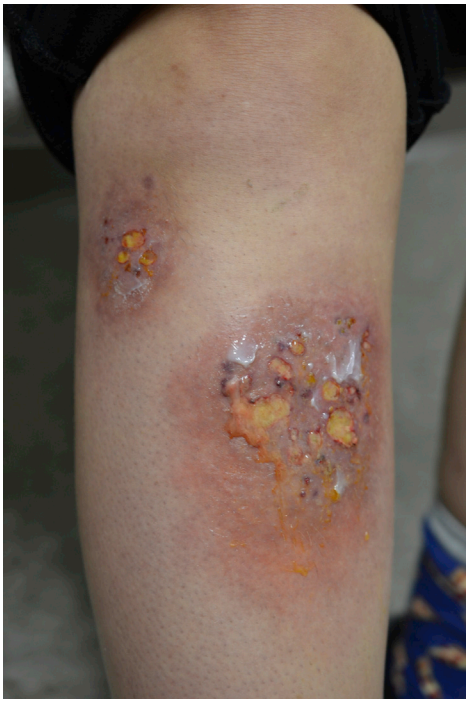

Fig 2. A young woman with cribriform ulceration, which progressed rapidly over her shins, and with new bloody diarrhea and abdominal pain.

Indirect immunofluorescence

For the analysis of ANCA by immunofluorescence, patient serum is incubated with ethanol-fixed neutrophils.[31] Fluorescein-labeled IgG is added and the slide is viewed under a fluorescence microscope to identify the staining pattern and titer. There are 3 main types of ANCA fluorescent patterns: cytoplasmic (c-ANCA), perinuclear (p-ANCA), and atypical p-ANCA.[32,33] Positive c-ANCA is characterized by diffuse fluorescence throughout the cytoplasm and is most often associated with PR3 antibodies. Perinuclear staining with nuclear extension is seen with p-ANCA, which is most often associated with MPO antibodies. Atypical p-ANCA describes perinuclear staining without nuclear extension, often seen with antibodies against other neutrophil antigens, such as catalase, elastase, and lysozyme. ANCA results are considered uninterpretable if ANA is positive, as ANA can interfere with the interpretation of the p-ANCA pattern.[32]

c-ANCA indirect immunofluorescence has a sensitivity and specificity of 65%-77% and 97%-98% respectively for granulomatosis with polyangiitis.[34,35] Sensitivity is higher for p-ANCA in diagnosing microscopic polyangiitis, 85%-89%, but with slightly lower specificity of 81%-96%.[34,35] As with ANA testing, immunofluorescence is a time-consuming, labor-intensive process and subject to discrepancies between laboratories.[34]

Antigen-specific immunoassays for proteinase 3 and myeloperoxidase

Many commercial immunoassays are available for the detection of proteinase 3 (PR3) and myeloperoxidase (MPO).[35] Receiver operating characteristic curves show that the newest generation of antigen-specific assays offer improved sensitivity and specificity compared to immunofluorescence (Table III).[34] There is less inter-laboratory variability with antigen-specific assays.[34]

Unique situations for interpretation

Discordant immunoassay and immunofluorescence tests most often are associated with non-vasculitic autoimmune conditions, such as SLE, autoimmune arthritis, and IBD.[36] Similarly, atypical p-ANCA is much less likely to be associated with systemic vasculitis compared to classic p-ANCA and is more often seen in other inflammatory conditions, notably ulcerative colitis.[32]

Recommended approach to ordering

It is recommended to test for ANCA in cases of suspected small- or medium-vessel disease, such as in patients presenting with palpable or retiform purpura, papulonecrotic extremity lesions, or atypical ulcerations with purpura. Testing can also be ordered in combination with other diagnostic tests to provide evidence for IBD.[37,38] If using a gating strategy for ANCA-associated vasculitis, the newest recommendations are to employ an initial screening test with PR3 and MPO autoantibodies, then reflex to immunofluorescence, if positive.[35] Many laboratories order autoantibodies and immunofluorescence at the same time, which is a reasonable strategy. However, if an immunofluorescence-first gating strategy has been performed, it is important to recognize improved sensitivity and specificity with ordering PR3 and MPO autoantibodies. Additionally, ANCA titers have been shown to correlate with disease activity, but the use of serial measurements to predict relapse remains controversial with only modest value for predicting a disease flare as a single test.[39-41]

DERMATOMYOSITIS AUTOANTIBODY TESTING

Scenario: A 55-year-old woman presents with progressive weakness, dyspnea, painful keratotic papules, and shallow ulcerations on her palms. A myositis panel is ordered and is positive for anti-MDA-5. With the presence of this particular antibody, what is the expected phenotype of dermatomyositis and what additional testing is indicated?

Table IV. Dermatomyositis autoantibodies included on a commercially available panel and their associated clinical phenotypes

Autoantibody	Clinical associations
Anti-Jo1	Associated with antisynthetase syndrome, characterized by the triad of arthritis, myositis, and interstitial lung disease.[42,43] Patients present with classic skin lesions, including Gottron papules and heliotrope rash.[44]
Anti-Mi2	Associated with classic skin lesions including Gottron papules, heliotrope rash, shawl sign, and photosensitivity.[45-48] Symmetric proximal muscle involvement can be severe but is typically responsive to corticosteroids.[49] Prognosis is generally good, with low risk of malignancy or interstitial lung disease.
Anti-TIF1 (previously anti-P155/140)	The most common MSA found among Caucasian patients, with lower incidence in Asian populations.[50] Anti-TIF1 is associated with severe skin involvement, including diffuse photoerythema, psoriasiform lesions, palmar hyperkeratosis, and verruca-like papules.[51,52] It is a diagnostic indicator of increased malignancy risk, with 78% sensitivity for cancer in adults with dermatomyositis.[53-55]
Anti-MDA5 (previously CADM-140)	Usually associated with clinically amyopathic disease with presentations that include mucocutaneous ulcerative lesions, palmar papules, panniculitis, and diffuse alopecia.[56,57] Patients with anti-MDA5 have increased risk of rapidly progressive interstitial lung disease, particularly when cutaneous ulceration is present.[58-60]
Anti-NXP2	Seen more often in juvenile dermatomyositis with the unique cutaneous manifestation of calcinosis cutis, severe myopathy, and poor prognosis.[61-63] Adults with anti-NXP2 typically present with classic cutaneous findings and have increased risk for malignancy.[54,61]
Anti-SAE	Associated with severe adult-onset dermatomyositis with classic skin manifestations preceding myositis.[64,65] Diffuse dark-red rash with ulceration has been observed in a Chinese cohort.[66]

anti-MDA5, Anti-melanoma differentiation-associated gene 5.

Key points

- Myositis-specific antibodies (MSA) have utility in the initial diagnosis of dermatomyositis and classification of patients into clinically relevant subsets.
- The MSA profile associated with each subset can be used to guide appropriate work up for malignancy and associated systemic disease.
- Scenario interpretation: Anti-MDA-5 pattern dermatomyositis frequently demonstrates acral papules and ulcerations and rapidly progressive interstitial lung disease. Pulmonary function testing should be performed without delay.

MSAs are antibodies exclusively associated with idiopathic inflammatory myopathy, including dermatomyositis and polymyositis. Many of these antibodies have been associated with unique clinical features, providing information on disease prognosis and anticipated systemic complications. Commercially available dermatomyositis test panels use immunologic methods of immunoprecipitation and ELISA to detect individual MSAs. MSAs associated with dermatomyositis that are typically included on commercial panels are described in Table IV[42-66]; however, as research is ongoing for these phenotypes, they are not labeled by sensitivity or specificity.

Recommended approach to ordering

The role of MSA testing in diagnosis and management of dermatomyositis continues to be debated, given recent advancement and availability of testing.[67,68] The latest guidelines from the European League Against Rheumatism and the American College of Rheumatology only included anti-Jo-1 in their classification criteria, but other MSAs were not widely available at that time.[69] For the medical dermatologist, these autoantibodies may show value in confirming subset diagnoses, such as the MDA-5 or Jo-1 phenotypes, even as debates continue on their value for utility, prognosis, and cancer screening.[50,58,70,71]

AUTOANTIBODY TESTING FOR BLISTERING DISEASES

Scenario: A 52-year-old woman is found to have positive low titers for ELISA BP180 and is referred for suspected diagnosis of bullous pemphigoid. However, her clinical presentation demonstrates only a single large bulla. Does this confirm bullous pemphigoid (Fig 3)?

Key points

- Antibody testing can facilitate the diagnosis of autoimmune blistering diseases and characterize disease activity.
- Scenario interpretation: The test does not confirm bullous pemphigoid. Low titer results for BP180 and BP230 do not show a high level of specificity for bullous pemphigoid and should be confirmed through direct immunofluorescence (DIF). This patient had an edema bulla.

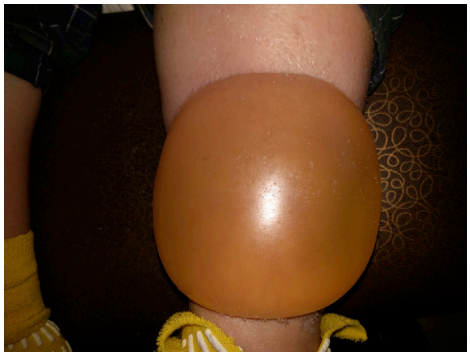

Fig 3. A middle-aged woman with a single large bulla over the lower extremity. She had no pruritus and no previous bullae and only presented after a slightly elevated bullous pemphigoid antibody test returned on testing from a recent hospitalization for congestive heart failure.

Bullous pemphigoid antibody testing

Commercially available ELISAs detect 2 antibodies associated with bullous pemphigoid, BP180 and BP230. BP180 has sensitivity of 54%-95% and specificity of 90%-99% when high titers of BP180 are present.[72-79] Sensitivity that delineates bullous pemphigoid from mucous membrane pemphigoid (MMP) is at the higher end of the above range, as MMP is often not detected by BP180 ELISA assays and other antigens may be causative in MMP.[73,75] BP230 has sensitivity of 48%-82% and specificity of 65%-99% with a weak added diagnostic value compared to testing for BP180 alone.[72,73,76,78-80] Specificity is reduced with lower titers of BP180 and BP230, between 10 and 20 U/mL, which can be seen in a range of other dermatoses.[81,82] In comparison, indirect immunofluorescence (IIF) has sensitivity of 73%-76% and specificity of 96%-100% for bullous pemphigoid,[73] and the gold standard, DIF, has sensitivity and specificity of 91%-96% and 98%, respectively.[73,83] Additionally, BP180 antibody titers correlate with disease activity and may be predictive of disease flares.[74,84,85]

Pemphigus antibody testing

Commercially available ELISAs detect desmoglein 1 antibodies associated with pemphigus vulgaris and pemphigus foliaceus and desmoglein 3 antibodies associated with pemphigus vulgaris. Desmoglein 1 has sensitivity of 92%-98% and specificity of 98%-100% for pemphigus foliaceus.[86-89] Desmoglein 3 has similar characteristics for pemphigus vulgaris, with sensitivity of 94%-100% and specificity of 98%-100%.[86-89] Desmoglein 1 and 3 ELISAs have been shown to be more sensitive than IIF,[86,90] which has sensitivity of 79%-84%,[87] while also providing a more objective and less labor-intensive test. The gold standard, DIF, has sensitivity of 94%-100%.[91,92] Antibody titers, particularly desmoglein 1, correlate with disease activity.[87,90,93]

Epidermolysis bullosa acquisita antibody testing

Commercially available ELISAs that detect collagen 7 have sensitivity of 86%-99%, with specificity of 98%-99% for epidermolysis bullosa acquisita.[94-97] In comparison, IIF has sensitivity of 92%-100% and specificity of 98%-100% for distinguishing between pemphigoid diseases when salt-split skin is used as the substrate.[94-98]

Although highly sensitive, IIF alone is not sufficient to distinguish epidermolysis bullosa acquisita from bullous pemphigoid, because linear deposits of IgG at the dermal-epidermal junction are seen in both diseases. Distinction between the 2 diagnoses can be made using salt-split skin or by identifying collagen 7 autoantibodies.[97,99] Additionally, collagen 7 antibody levels correlate with disease activity.[94,95] Collagen 7 antibodies can be seen in bullous SLE, but this diagnosis can be distinguished by clinical presentation, histology, and concomitant diagnosis of SLE.[99,100]

Dermatitis herpetiformis antibody testing

Testing for IgA anti-tissue transglutaminase (tTG) is performed by widely available commercial ELISA with sensitivity of 47%-95% and specificity 98%-100%.[101-103] ELISA for epidermal transglutaminase antibodies (anti-eTG) has similar characteristics, with sensitivity of 52%-100% and specificity of 93%-100%.[101-103] Endomysial antibodies (EMA) are detected via IIF assay with sensitivity of 52%-100% and specificity approaching 100%.[101] This assay is time-consuming and labor-intensive compared to ELISA assays. In comparison, the gold standard, DIF, has sensitivity and specificity approaching 100%.[101] IIF has limited utility for dermatitis herpetiformis. Although antibody testing is less sensitive than DIF, it is relatively quick and inexpensive, and titers correlate with bowel disease activity.[102,104] Additionally, antibody titers diminish with implementation of a gluten-free diet and may be useful for monitoring dietary adherence.[102]

Recommended approach to ordering

The DIF still stands as the gold standard for all blistering diseases.[91] However, serologic testing can be used as an adjunct to facilitate diagnosis when biopsies cannot be performed and in equivocal situations. Antibody testing is highly sensitive and specific for pemphigus, epidermolysis bullosa, bullous pemphigoid, and dermatitis

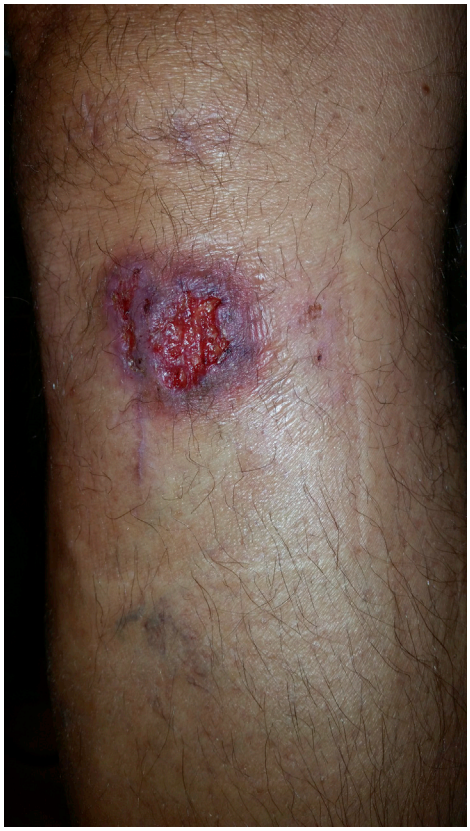

Fig 4. A young man with an irregular ulceration with violaceous borders and some involvement of a previous scar.

herpetiformis.[73,90,97] However, bullous pemphigoid antibodies have poor sensitivity for MMP, and low titers may be seen in other dermatologic conditions.[73,75] Further, antibody testing for autoimmune blistering disorders provides information on disease activity; however, the frequency of these tests for routine monitoring has not been established.

INFLAMMATORY BOWEL DISEASE LABORATORY SCREENING

Scenario: A 25-year-old man presents with a large, painful ulcer of his left shin that has been enlarging rapidly. Physical examination reveals a full-thickness ulcer with irregular violaceous borders and surrounding erythema. He has not followed up despite multiple attempts to schedule him for a colonoscopy. What laboratory tests can be ordered to support whether the pyoderma gangrenosum is associated with IBD (Fig 4)?

Key points

- Fecal, molecular, and serologic tests can be useful in differentiating between Crohn disease and ulcerative colitis.
- Scenario interpretation: Although multiple tests are available and associated with IBD, the fecal calprotectin offers the highest sensitivity and specificity in combination with the lowest cost.

Several cutaneous and mucosal disorders, including pyoderma gangrenosum, erythema nodosum, Sweet syndrome, aphthous stomatitis, and pyostomatitis vegetans, are strongly associated with IBD,[105,106] raising suspicion for underlying disease. Although endoscopy is the gold standard for diagnosing IBD, there are many limitations and situations in which patients cannot obtain the test or the test may be nondiagnostic.[107]

Anti-saccharomyces cerevisiae antibodies

Anti-saccharomyces cerevisiae antibodies (ASCAs) have been identified as a serologic marker for Crohn disease,[108] preceding diagnosis in roughly a third of cases. They are associated with younger age of diagnosis, development of strictures, and ileal involvement.[109]

IgG ASCA ELISA has sensitivity of 41%-84% and specificity of 86%-96%.[108,110,111] IgA ASCA ELISA has sensitivity of 34%-84% and specificity of 84%-95%.[108,110,111] Testing for both IgG and IgA ASCAs increases sensitivity to 51%-92%.[108,110,111] Detection of positive ASCA and negative p-ANCA increases sensitivity to 55%, with specificity of 93% for Crohn disease.[112]

Inflammatory bowel disease differentiation panels

IBD differentiation panels are commercially available and typically include a combination of serologic, genetic, and inflammatory markers. One nationally available panel includes 8 serologic markers, including ASCAs and ANCA, 4 genetic single nucleotide polymorphisms, and 5 inflammatory markers.[113] Serologic markers are tested via ELISA with the exception of p-ANCA, which is assessed by IIF.

This test has a sensitivity of 89% and specificity of 81% for the diagnosis of Crohn disease.[113] Diagnostic performance is slightly higher for ulcerative colitis, with sensitivity of 98% and specificity 84%.[113] Although receiver operating characteristic curve analysis is very strong for diagnosis and differentiation, the test is notably expensive.

Fecal calprotectin

Fecal calprotectin is a neutrophilic protein that leaks into the intestines with intestinal inflammation, as seen in colitis, leading to deposition of calprotectin in feces. Calprotectin has been found to correlate

with disease activity in IBD[114] and is stable in feces for about 7 days.

Commercially available fecal calprotectin ELISA has sensitivity of 93% and specificity of 96% for IBD in adults compared to the gold standard, endoscopy.[115] Similar test characteristics are seen in children, with sensitivity and specificity of 76% and 92%, respectively.[115] Fecal calprotectin has shown utility as a screening test to determine the need for endoscopy. In adults with suspected IBD and pretest probability of 32%, abnormal fecal calprotectin increased probability to 91%, whereas normal results reduced probability to 3%, negating the need for an urgent endoscopy.[115] This test will not differentiate ulcerative colitis from Crohn disease, and it is important to note that colon cancer could also create a positive result.

Recommended approach to ordering

Although endoscopy remains the gold standard in patients with pyoderma gangrenosum and other diseases associated with IBD, in vitro diagnostics can be used to determine the urgency for endoscopy and to test for activity in patients with a known history of IBD, and can be considered for those in whom a colonoscopy cannot be performed. Of the tests available, the fecal calprotectin test offers an extremely high sensitivity and specificity while being inexpensive, especially compared to the serologic and molecular differentiation panels.

CONCLUSIONS

Laboratory testing, when ordered appropriately, can facilitate the diagnosis of dermatologic autoimmune diseases. Results are to be interpreted in the context of clinical presentation. Negative results in the setting of high suspicion should prompt reflection on the selected tests and their associated characteristics.

Conflicts of interest

None disclosed.

REFERENCES

1. Sohn KY, Khan WI. ANA testing from microscopy to multiplexing. *Clinical Laboratory News*. AACC. June 1, 2014. Accessed September 1, 2020. Available at: https://www.aacc.org/cln/articles/2014/june/ana-testing
2. Chan EK, Damoiseaux J, Carballo OG, et al. Report of the first international consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns 2014–2015. *Front Immunol*. 2015;6:412.
3. Chan EK, Damoiseaux J, de Melo Cruvinel W, et al. Report on the second International Consensus on ANA Pattern (ICAP) workshop in Dresden 2015. *Lupus*. 2016;25(8):797-804.
4. Damoiseaux J, Andrade LEC, Carballo OG, et al. Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective. *Ann Rheum Dis*. 2019;78(7):879-889.
5. Kang I, Siperstein R, Quan T, Breitenstein ML. Utility of age, gender, ANA titer and pattern as predictors of anti-ENA and -dsDNA antibodies. *Clin Rheum*. 2004;23(6):509-515.
6. Didier K, Bolko L, Giusti D, et al. Autoantibodies associated with connective tissue diseases: what meaning for clinicians? *Front Immunol*. 2018;9:541.
7. El-Chennawi FA, Mosaad YM, Habib HM, El-Degheidi T. Comparative study of antinuclear antibody detection by indirect immunofluorescence and enzyme immunoassay in lupus patients. *Immunol Invesigt*. 2009;38(8):839-850.
8. Tonutti E, Bassetti D, Piazza A, et al. Diagnostic accuracy of ELISA methods as an alternative screening test to indirect immunofluorescence for the detection of antinuclear antibodies. Evaluation of five commercial kits. *Autoimmunity*. 2004;37(2):171-176.
9. Abeles AM, Abeles M. The clinical utility of a positive antinuclear antibody test result. *Am J Med*. 2013;126(4):342-348.
10. Rigon A, Infantino M, Merone M, et al. The inter-observer reading variability in anti-nuclear antibodies indirect (ANA) immunofluorescence test: a multicenter evaluation and a review of the literature. *Autoimmun Rev*. 2017;16(12):1224-1229.
11. Pisetsky DS. Antinuclear antibody testing—Misunderstood or misbegotten? *Nat Rev Rheumatol*. 2017;13(8):495-502.
12. Martins TB, Burlingame R, von Mühlen CA, Jaskowski TD, Litwin CM, Hill HR. Evaluation of multiplexed fluorescent microsphere immunoassay for detection of autoantibodies to nuclear antigens. *Clin Diagn Lab Immunol*. 2004;11(6):1054-1059.
13. Nifli A-P, Notas G, Mamoulaki M, et al. Comparison of a multiplex, bead-based fluorescent assay and immunofluorescence methods for the detection of ANA and ANCA autoantibodies in human serum. *J Immunol Methods*. 2006;311(1-2):189-197.
14. ANA multiplex with reflex to 11 antibody cascade. QuestDiagnostics. Accessed October 12, 2020. Available at: https://testdirectory.questdiagnostics.com/test/test-detail/19946/ana-multiplex-with-reflex-to-11-antibody-cascade?cc=MASTER
15. Antinuclear antibodies (ANA) Profile, 9-biomarkers, by Multiplex Immunoassay, dsDNA, RNP, Sm, SS-A, SS-B, Scl-70, Chromatin, Jo-1, Centromere B. LabCorp; 2020.
16. Mulhearn B, Tansley SL, McHugh NJ. Autoantibodies in connective tissue disease. *Best Pract Res Clin Rheumatol*. 2020;34(1):101462.
17. Linnik MD, Hu JZ, Heilbrunn KR, et al. Relationship between anti–double-stranded DNA antibodies and exacerbation of renal disease in patients with systemic lupus erythematosus. *Arthritis Rheum*. 2005;52(4):1129-1137.
18. Franceschini F, Cavazzana I. Anti-Ro/SSA and La/SSB antibodies. *Autoimmunity*. 2005;38:55-63.
19. Shovman O, Gilburd B, Zandman-Goddard G, Yehiely A, Langevitz P, Shoenfeld Y. Multiplexed AtheNA multi-lyte immunoassay for ANA screening in autoimmune diseases. *Autoimmunity*. 2005;38(1):105-109.
20. Biagini RE, Parks CG, Smith JP, Sammons DL, Robertson SA. Analytical performance of the AtheNA MultiLyte ANA II assay in sera from lupus patients with multiple positive ANAs. *Anal Bioanal Chem*. 2007;388(3):613-618.
21. Hanly JG, Thompson K, McCurdy G, Fougere L, Theriault C, Wilton K. Measurement of autoantibodies using multiplex methodology in patients with systemic lupus erythematosus. *J Immunol Methods*. 2010;352(1-2):147-152.

22. Binder SR. Autoantibody detection using multiplex technologies. *Lupus*. 2006;15(7):412-421.
23. Op De Beéck KO, Vermeersch P, Verschuren P, et al. Antinuclear antibody detection by automated multiplex immunoassay in untreated patients at the time of diagnosis. *Autoimmun Rev*. 2012;12(2):137-143.
24. Position statement: methodology of testing for antinuclear antibodies. American College of Rheumatology. Accessed. Available at:, 2015. Accessed. Available at: https://www.rheumatology.org/Portals/0/Files/Methodology%20of%20Testing%20Antinuclear%20Antibodies%20Position%20Statement.pdf
25. Naides SJ, Genzen JR, Abel G, Bashleben C, Ansari MQ. Antinuclear antibodies (ANA) testing method variability: a survey of participants in the College of American Pathologists'(CAP) Proficiency Testing Program. *J Rheumatol*. 2020;47(12):1768-1773.
26. Buzzulini F, Rigon A, Soda P, et al. The classification of Crithidia luciliae immunofluorescence test (CLIFT) using a novel automated system. *Arthritis Res Ther*. 2014;16(2):R71.
27. Yazdany J, Schmajuk G, Robbins M, et al. Choosing wisely: the American College of Rheumatology's Top 5 list of things physicians and patients should question. *Arthritis Care Res*. 2013;65(3):329-339.
28. Fritzler MJ. Choosing wisely: review and commentary on antinuclear antibody (ANA) testing. *Autoimmun Rev*. 2016;15(3):272-280.
29. Li H, Lin S, Yang S, Chen L, Zheng X. Diagnostic value of serum complement C3 and C4 levels in Chinese patients with systemic lupus erythematosus. *Clin Rheumatol*. 2015;34(3):471-477.
30. Narayanan K, Marwaha V, Shanmuganandan K, Shankar S. Correlation between systemic lupus erythematosus disease activity index, C3, C4 and anti-dsDNA antibodies. *Med J Armed Forces India*. 2010;66(2):102-107.
31. Cohen Tervaert JWC, Damoiseaux J. Antineutrophil cytoplasmic autoantibodies: how are they detected and what is their use for diagnosis, classification and follow-up? *Clin Rev Allergy Immunol*. 2012;43(3):211-219.
32. Perel SB, Prain KM, Wilson RJ, Hogan PG, Gillis D, Wong RC. Diagnostic value of distinguishing and reporting different perinuclear ANCA (P-ANCA) immunofluorescence patterns: a prospective study. *Am J Clin Pathol*. 2013;140(2):184-192.
33. Savige J, Paspaliaris B, Silvestrini R, et al. A review of immunofluorescent patterns associated with antineutrophil cytoplasmic antibodies (ANCA) and their differentiation from other antibodies. *J Clin Pathol*. 1998;51(8):568-575.
34. Damoiseaux J, Csernok E, Rasmussen N, et al. Detection of antineutrophil cytoplasmic antibodies (ANCAs): a multicentre European Vasculitis Study Group (EUVAS) evaluation of the value of indirect immunofluorescence (IIF) versus antigen-specific immunoassays. *Ann Rheum Dis*. 2017;76(4):647-653.
35. Bossuyt X, Cohen Tervaert JW, Arimura Y, et al. Position paper: revised 2017 International consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. *Nat Rev Rheumatol*. 2017;13(11):683-692.
36. Rao DA, Wei K, Merola JF, et al. Myeloperoxidase-antineutrophil cytoplasmic antibodies (MPO-ANCA) and proteinase 3-ANCA without immunofluorescent ANCA found by routine clinical testing. *J Rheumatol*. 2015;42(5):847-852.
37. Marzano AV, Raimondo MG, Berti E, Meroni PL, Ingegnoli F. Cutaneous manifestations of ANCA-associated small vessels vasculitis. *Clin Rev Allergy Immunol*. 2017;53(3):428-438.
38. Chen KR. Skin involvement in ANCA-associated vasculitis. *Clin Exp Nephrol*. 2013;17(5):676-682.
39. Tomasson G, Grayson PC, Mahr AD, LaValley M, Merkel PA. Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis—a meta-analysis. *Rheumatology*. 2012;51(1):100-109.
40. Kemna MJ, Damoiseaux J, Austen J, et al. ANCA as a predictor of relapse: useful in patients with renal involvement but not in patients with nonrenal disease. *J Am Soc Nephrol*. 2015;26(3):537-542.
41. Han WK, Choi HK, Roth RM, McCluskey RT, Niles JL. Serial ANCA titers: useful tool for prevention of relapses in ANCA-associated vasculitis. *Kidney Int*. 2003;63(3):1079-1085.
42. Zamora AC, Hoskote SS, Abascal-Bolado B, et al. Clinical features and outcomes of interstitial lung disease in anti-Jo-1 positive antisynthetase syndrome. *Respir Med*. 2016;118:39-45.
43. Imbert-Masseau A, Hamidou M, Agard C, Grolleau JY, Chérin P. Antisynthetase syndrome. *Joint Bone Spine*. 2003;70(3):161-168.
44. Matsushita T, Hasegawa M, Fujimoto M, et al. Clinical evaluation of anti-aminoacyl tRNA synthetase antibodies in Japanese patients with dermatomyositis. *J Rheumatol*. 2007;34(5):1012-1018.
45. Cruellas MGP, Viana VdST, Levy-Neto M, Souza FHCd, Shinjo SK. Myositis-specific and myositis-associated autoantibody profiles and their clinical associations in a large series of patients with polymyositis and dermatomyositis. *Clinics (Sao Paulo)*. 2013;68(7):909-914.
46. Targoff IN, Reichlin M. The association between Mi-2 antibodies and dermatomyositis. *Arthritis Rheum*. 1985;28(7):796-803.
47. Love LA, Leff RL, Fraser DD, et al. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. *Medicine (Baltimore)*. 1991;70(6):360-374.
48. Satoh M, Tanaka S, Ceribelli A, Calise SJ, Chan EK. A comprehensive overview on myositis-specific antibodies: new and old biomarkers in idiopathic inflammatory myopathy. *Clin Rev Allergy Immunol*. 2017;52(1):1-19.
49. Pinal-Fernandez I, Mecoli CA, Casal-Dominguez M, et al. More prominent muscle involvement in patients with dermatomyositis with anti-Mi2 autoantibodies. *Neurology*. 2019;93(19):e1768-e1777.
50. Tartar DM, Chung L, Fiorentino DF. Clinical significance of autoantibodies in dermatomyositis and systemic sclerosis. *Clin Dermatol*. 2018;36(4):508-524.
51. Ueda-Hayakawa I, Hamaguchi Y, Okiyama N, et al. Autoantibody to transcriptional intermediary factor-1β as a myositis-specific antibody: clinical correlation with clinically amyopathic dermatomyositis or dermatomyositis with mild myopathy. *Br J Dermatol*. 2019;180(4):881-887.
52. Fiorentino DF, Kuo K, Chung L, Zaba L, Li S, Casciola-Rosen L. Distinctive cutaneous and systemic features associated with antitranscriptional intermediary factor-1γ antibodies in adults with dermatomyositis. *J Am Acad Dermatol*. 2015;72(3):449-455.
53. Shimizu K, Kobayashi T, Kano M, Hamaguchi Y, Takehara K, Matsushita T. Anti-transcriptional intermediary factor 1-γ antibody as a biomarker in patients with dermatomyositis. *J Dermatol*. 2020;47(1):64-68.
54. Fiorentino DF, Chung LS, Christopher-Stine L, et al. Most patients with cancer-associated dermatomyositis have antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1γ. *Arthritis Rheum*. 2013;65(11):2954-2962.
55. Trallero-Araguás E, Rodrigo-Pendás JÁ, Selva-O'Callaghan A, et al. Usefulness of anti-p155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and meta-analysis. *Arthritis Rheum*. 2012;64(2):523-532.

56. Kurtzman DJB, Vleugels RA. Anti-melanoma differentiation–associated gene 5 (MDA5) dermatomyositis: a concise review with an emphasis on distinctive clinical features. *J Am Acad Dermatol*. 2018;78(4):776-785.
57. Fiorentino D, Chung L, Zwerner J, Rosen A, Casciola-Rosen L. The mucocutaneous and systemic phenotype of dermatomyositis patients with antibodies to MDA5 (CADM-140): a retrospective study. *J Am Acad Dermatol*. 2011;65(1):25-34.
58. DeWane ME, Waldman R, Lu J. Dermatomyositis: clinical features and pathogenesis. *J Am Acad Dermatol*. 2020;82(2):267-281.
59. Sontheimer RD. MDA5 autoantibody—Another indicator of clinical diversity in dermatomyositis. *Ann Transl Med*. 2017;5(7):160.
60. Narang NS, Casciola-Rosen L, Li S, Chung L, Fiorentino DF. Cutaneous ulceration in dermatomyositis: association with anti-melanoma differentiation–associated gene 5 antibodies and interstitial lung disease. *Arthritis Care Res*. 2015;67(5):667-672.
61. Albayda J, Pinal-Fernandez I, Huang W, et al. Dermatomyositis patients with anti-nuclear matrix protein-2 autoantibodies have more edema, more severe muscle disease, and increased malignancy risk. *Arthritis Care Res*. 2017;69(11):1771-1776.
62. Aouizerate J, De Antonio M, Bader-Meunier B, et al. Muscle ischaemia associated with NXP2 autoantibodies: a severe subtype of juvenile dermatomyositis. *Rheumatology*. 2018;57(5):873-879.
63. Tansley SL, Betteridge ZE, Shaddick G, et al. Calcinosis in juvenile dermatomyositis is influenced by both anti-NXP2 autoantibody status and age at disease onset. *Rheumatology*. 2014;53(12):2204-2208.
64. Bodoki L, Nagy-Vincze M, Griger Z, Betteridge Z, Szöllősi L, Dankó K. Four dermatomyositis-specific autoantibodies-anti-TIF1γ, anti-NXP2, anti-SAE and anti-MDA5-in adult and juvenile patients with idiopathic inflammatory myopathies in a Hungarian cohort. *Autoimmun Rev*. 2014;13(12):1211-1219.
65. Betteridge ZE, Gunawardena H, Chinoy H, et al. Clinical and human leucocyte antigen class II haplotype associations of autoantibodies to small ubiquitin-like modifier enzyme, a dermatomyositis-specific autoantigen target, in UK Caucasian adult-onset myositis. *Ann Rheum Dis*. 2009;68(10):1621-1625.
66. Ge Y, Lu X, Shu X, Peng Q, Wang G. Clinical characteristics of anti-SAE antibodies in Chinese patients with dermatomyositis in comparison with different patient cohorts. *Sci Rep*. 2017;7(1):188.
67. Lundberg IE, Tjärnlund A. Response to: '2017 EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups: little emphasis on autoantibodies, why?' by Malaviya. *Ann Rheum Dis*. 2018;77(11):e78.
68. Malaviya AN. 2017 EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups: little emphasis on autoantibodies, why? *Ann Rheum Dis*. 2018;77(11):e77.
69. Lundberg IE, Tjärnlund A, Bottai M, et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann Rheum Dis*. 2017;76(12):1955-1964.
70. Waldman R, DeWane ME, Lu J. Dermatomyositis: diagnosis and treatment. *J Am Acad Dermatol*. 2020;82(2):283-296.
71. Gono T, Kuwana M. Current understanding and recent advances in myositis-specific and -associated autoantibodies detected in patients with dermatomyositis. *Expert Rev Clin Immunol*. 2020;16(1):79-89.
72. Keller JJ, Kittridge AL, Debanne SM, Korman NJ. Evaluation of ELISA testing for BP180 and BP230 as a diagnostic modality for bullous pemphigoid: a clinical experience. *Arch Dermatol Res*. 2016;308(4):269-272.
73. Sárdy M, Kostaki D, Varga R, Peris K, Ruzicka T. Comparative study of direct and indirect immunofluorescence and of bullous pemphigoid 180 and 230 enzyme-linked immunosorbent assays for diagnosis of bullous pemphigoid. *J Am Acad Dermatol*. 2013;69(5):748-753.
74. Kobayashi M, Amagai M, Kuroda-Kinoshita K, et al. BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid. *J Dermatol Sci*. 2002;30(3):224-232.
75. Zillikens D, Mascaro JM, Rose PA, et al. A highly sensitive enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. *J Invest Dermatol*. 1997;109(5):679-683.
76. Lee EH, Kim YH, Kim S, Kim SE, Kim SC. Usefulness of enzyme-linked immunosorbent assay using recombinant BP180 and BP230 for serodiagnosis and monitoring disease activity of bullous pemphigoid. *Ann Dermatol*. 2012;24(1):45-55.
77. Sitaru C, Dähnrich C, Probst C, et al. Enzyme-linked immunosorbent assay using multimers of the 16th non-collagenous domain of the BP180 antigen for sensitive and specific detection of pemphigoid autoantibodies. *Exp Dermatol*. 2007;16(9):770-777.
78. Thoma-Uszynski S, Uter W, Schwietzke S, et al. BP230-and BP180-specific auto-antibodies in bullous pemphigoid. *J Invest Dermatol*. 2004;122(6):1413-1422.
79. Charneux J, Lorin J, Vitry F, et al. Usefulness of BP230 and BP180-NC16a enzyme-linked immunosorbent assays in the initial diagnosis of bullous pemphigoid: a retrospective study of 138 patients. *Arch Dermatol*. 2011;147(3):286-291.
80. Yoshida M, Hamada T, Amagai M, et al. Enzyme-linked immunosorbent assay using bacterial recombinant proteins of human BP230 as a diagnostic tool for bullous pemphigoid. *J Dermatol Sci*. 2006;41(1):21-30.
81. Wang M, Lehman JS, Camilleri MJ, Drage LA, Wieland CN. Circulating bullous pemphigoid autoantibodies in the setting of negative direct immunofluorescence findings for bullous pemphigoid: a single-center retrospective review. *J Am Acad Dermatol*. 2019;81(2):472-479.
82. Liu Z, Chen L, Zhang C, Xiang LF. Circulating bullous pemphigoid 180 autoantibody can be detected in a wide spectrum of patients with other dermatologic conditions: a cross-sectional study. *J Am Acad Dermatol*. 2019;80(3):774-775.
83. Mysorekar VV, Sumathy T, Shyam Prasad AL. Role of direct immunofluorescence in dermatological disorders. *Indian Dermatol Online J*. 2015;6(3):172-180.
84. Cai S, Lim YL, Li W, et al. Anti-BP180 NC16A IgG titres as an indicator of disease activity and outcome in Asian patients with bullous pemphigoid. *Ann Acad Med Singap*. 2015;44(4):119-126.
85. Schmidt E, Obe K, Bröcker EB, Zillikens D. Serum levels of autoantibodies to BP180 correlate with disease activity in patients with bullous pemphigoid. *Arch Dermatol*. 2000;136(2):174-178.
86. Harman KE, Gratian MJ, Seed PT, Bhogal BS, Challacombe SJ, Black MM. Diagnosis of pemphigus by ELISA: a critical evaluation of two ELISAs for the detection of antibodies to the major pemphigus antigens, desmoglein 1 and 3. *Clin Exp Dermatol*. 2000;25(3):236-240.
87. Schmidt E, Dähnrich C, Rosemann A, et al. Novel ELISA systems for antibodies to desmoglein 1 and 3: correlation

of disease activity with serum autoantibody levels in individual pemphigus patients. *Exp Dermatol*. 2010;19(5):458-463.

88. Amagai M, Komai A, Hashimoto T, et al. Usefulness of enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3 for serodiagnosis of pemphigus. *Br J Dermatol*. 1999;140(2):351-357.
89. Ishii K, Amagai M, Hall RP, et al. Characterization of autoantibodies in pemphigus using antigen-specific enzyme-linked immunosorbent assays with baculovirus-expressed recombinant desmogleins. *J Immunol*. 1997;159(4):2010-2017.
90. Abasq C, Mouquet H, Gilbert D, et al. ELISA testing of anti—desmoglein 1 and 3 antibodies in the management of pemphigus. *Arch Dermatol*. 2009;145(5):529-535.
91. Buch AC, Kumar H, Panicker N, Misal S, Sharma Y, Gore CR. A cross-sectional study of direct immunofluorescence in the diagnosis of immunobullous dermatoses. *Indian J Dermatol*. 2014;59(4):364-368.
92. Sano SM, Quarracino MC, Aguas SC, et al. Sensitivity of direct immunofluorescence in oral diseases. Study of 125 cases. *Med Oral Patol Oral Cir Bucal*. 2008;13(5):E287-E291.
93. Harman KE, Seed PT, Gratian MJ, Bhogal BS, Challacombe SJ, Black MM. The severity of cutaneous and oral pemphigus is related to desmoglein 1 and 3 antibody levels. *Br J Dermatol*. 2001;144(4):775-780.
94. Marzano AV, Cozzani E, Fanoni D, et al. Diagnosis and disease severity assessment of epidermolysis bullosa acquisita by ELISA for anti-type VII collagen autoantibodies: an Italian multicentre study. *Br J Dermatol*. 2013;168(1):80-84.
95. Saleh MA, Ishii K, Kim YJ, et al. Development of NC1 and NC2 domains of type VII collagen ELISA for the diagnosis and analysis of the time course of epidermolysis bullosa acquisita patients. *J Dermatol Sci*. 2011;62(3):169-175.
96. Kim JH, Kim YH, Kim S, et al. Serum levels of anti-type VII collagen antibodies detected by enzyme-linked immunosorbent assay in patients with epidermolysis bullosa acquisita are correlated with the severity of skin lesions. *J Eur Acad Dermatol Venereol*. 2013;27(2):e224-e230.
97. Komorowski L, Müller R, Vorobyev A, et al. Sensitive and specific assays for routine serological diagnosis of epidermolysis bullosa acquisita. *J Am Acad Dermatol*. 2013;68(3):e89-e95.
98. van Beek N, Zillikens D, Schmidt E. Diagnosis of autoimmune bullous diseases. *J Dtsch Dermatol Ges*. 2018;16(9):1077-1091.
99. Woodley DT, Remington J, Chen M. Autoimmunity to type VII collagen: *epidermolysis bullosa acquisita*. *Clin Rev Allergy Immunol*. 2007;33(1-2):78-84.
100. Shirahama S, Furukawa F, Yagi H, Tanaka T, Hashimoto T, Takigawa M. Bullous systemic lupus erythematosus: detection of antibodies against noncollagenous domain of type VII collagen. *J Am Acad Dermatol*. 1998;38(5 Pt 2):844-848.
101. Antiga E, Caproni M. The diagnosis and treatment of dermatitis herpetiformis. *Clin Cosmet Investig Dermatol*. 2015;8:257-265.
102. Bolotin D, Petronic-Rosic V. Dermatitis herpetiformis: part II. Diagnosis, management, and prognosis. *J Am Acad Dermatol*. 2011;64(6):1027-1033.
103. Rose C, Armbruster FP, Ruppert J, Igl B-W, Zillikens D, Shimanovich I. Autoantibodies against epidermal transglutaminase are a sensitive diagnostic marker in patients with dermatitis herpetiformis on a normal or gluten-free diet. *J Am Acad Dermatol*. 2009;61(1):39-43.
104. Marietta EV, Camilleri MJ, Castro LA, Krause PK, Pittelkow MR, Murray JA. Transglutaminase autoantibodies in dermatitis herpetiformis and celiac sprue. *J Invest Dermatol*. 2008;128(2):332-335.
105. Timani S, Mutasim DF. Skin manifestations of inflammatory bowel disease. *Clin Dermatol*. 2008;26(3):265-273.
106. Greuter T, Navarini A, Vavricka SR. Skin manifestations of inflammatory bowel disease. *Clin Rev Allergy Immunol*. 2017;53(3):413-427.
107. Harewood GC, Mattek NC, Holub JL, Peters D, Lieberman DA. Variation in practice of ileal intubation among diverse endoscopy settings: results from a National endoscopic database. *Aliment Pharmacol Ther*. 2005;22(6):571-578.
108. Teml A, Kratzer V, Schneider B, et al. Anti-Saccharomyces cerevisiae antibodies: a stable marker for Crohn's disease during steroid and 5-aminosalicylic acid treatment. *Am J Gastroenterol*. 2003;98(10):2226-2231.
109. Rinaldi M, Perricone R, Blank M, Perricone C, Shoenfeld Y. Anti-Saccharomyces cerevisiae autoantibodies in autoimmune diseases: from bread baking to autoimmunity. *Clin Rev Allergy Immunol*. 2013;45(2):152-161.
110. Vermeire S, Joossens S, Peeters M, et al. Comparative study of ASCA (Anti—Saccharomyces cerevisiae antibody) assays in inflammatory bowel disease. *Gastroenterology*. 2001;120(4):827-833.
111. Klebl FH, Bataille F, Hofstädter F, Herfarth H, Schölmerich J, Rogler G. Optimising the diagnostic value of anti-Saccharomyces cerevisiae-antibodies (ASCA) in Crohn's disease. *Int J Colorectal Dis*. 2004;19(4):319-324.
112. Reese GE, Constantinides VA, Simillis C, et al. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol*. 2006;101(1):2410-2422.
113. Plevy S, Silverberg MS, Lockton S, et al. Combined serological, genetic, and inflammatory markers differentiate non-IBD, Crohn's disease, and ulcerative colitis patients. *Inflamm Bowel Dis*. 2013;19(6):1139-1148.
114. von Arnim U, Wex T, Ganzert C, Schulz C, Malfertheiner P. Fecal calprotectin: a marker for clinical differentiation of microscopic colitis and irritable bowel syndrome. *Clin Exp Gastroenterol*. 2016;9:97-103.
115. Van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ*. 2010;341:c3369.