

Periodic Acid-Schiff Staining Parallels the Immunoreactivity Seen By Direct Immunofluorescence in Autoimmune Skin Diseases

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Abstract

Background: In many countries and laboratories, techniques such as direct immunofluorescence (DIF) are not available for the diagnosis of skin diseases. Thus, these laboratories are limited in the full diagnoses of autoimmune skin diseases, vasculitis, and rheumatologic diseases. In our experience with these diseases and the patient's skin biopsies, we have noted a positive correlation between periodic acid-Schiff (PAS) staining and immunofluorescence patterns; however, these were just empiric observations. In the current study, we aim to confirm these observations, given the concept that the majority of autoantibodies are glycoproteins and should thus be recognized by PAS staining. **Aims:** To compare direct immunofluorescent and PAS staining, in multiple autoimmune diseases that are known to exhibit specific direct immunofluorescent patterns. **Materials and Methods:** We studied multiple autoimmune skin diseases: Five cases of bullous pemphigoid, five cases of pemphigus vulgaris, ten cases of cutaneous lupus, ten cases of autoimmune vasculitis, ten cases of lichen planus (LP), and five cases of cutaneous drug reactions (including one case of erythema multiforme). In addition, we utilized 45 normal skin control specimens from plastic surgery reductions. **Results:** We found a 98% positive correlation between DIF and PAS staining patterns over all the disease samples. **Conclusion:** We recommend that laboratories without access to DIF always perform PAS staining in addition to hematoxylin and eosin (H&E) staining, for a review of the reactivity pattern.

Keywords: Autoimmune vasculitis, bullous pemphigoid, cutaneous drug reactions, direct immunofluorescence (DIF), lupus erythematosus (LE), periodic acid-Schiff (PAS), periodic acid-Schiff staining, pemphigus vulgaris, skin autoimmune blistering diseases

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Introduction

The diagnosis of skin autoimmune blistering diseases, autoimmune vasculitis, and drug reactions requires clinical history and judgment combined with histopathologic data and often direct and/or indirect immunofluorescence (DIF, IIF) studies.^[1] Autoimmune diseases can affect almost any part of the body, including the heart, brain, nerves, muscles, skin, eyes, joints, lungs, kidneys, the digestive tract, and blood vessels. The

cardinal sign of an autoimmune disease is inflammation that can cause redness, heat, pain, and swelling. How an autoimmune disease affects a patient depends on what specific tissues are targeted. One autoimmune disease may affect multiple tissues. For example, systemic lupus erythematosus (LE) can affect the skin, joints, kidneys, heart, nerves, and blood vessels. Type I diabetes mellitus may affect the eyes, kidneys, and muscles. A combination

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of factors is probably causative in most autoimmune diseases, including genetic as well as environmental factors.^[2,3]

Periodic acid-Schiff (PAS) is a staining method used to detect polysaccharides, manifested as glycogen, glycoproteins, glycolipids, and mucin in tissues.^[4,5] In general, the PAS stain is recommended to be performed on tissues fixed with 10% neutral buffered formalin.^[4,5] Based on the fact that many autoantibodies and complement are indeed glycoproteins,^[4,5] we aimed to determine if a correlation of patterns was present between PAS and DIF staining in multiple autoimmune skin diseases.

Materials and Methods

We utilized archival biopsies. A case-control study design was used to compare DIF positivity *versus* PAS positivity. Both DIF and PAS samples were taken from the same patient and the skin area at the same time.

All samples were reviewed via H&E and PAS staining; our staining was performed as previously described.^[6] In brief, we studied 45 archival skin biopsies; each biopsy was independently diagnosed by one board-certified dermatopathologist and one immunodermatologist, both in the USA. All biopsies were initially fixed in 10% buffered formalin, embedded in paraffin, and cut at 4 micron thicknesses. Our study included only the lesions that had classic histologic features of each disease. The Georgia Dermatopathology Associates Research Ethics Committee approved the study. A signed consent was obtained from the patients, and no patient identifiers were published. We studied ten cases of pemphigus vulgaris, five of bullous pemphigoid, ten cases of LE, ten of autoimmune vasculitis, ten cases of lichen planus (LP), and five cases of cutaneous drug reactions (including one of erythema multiforme). We utilized 45 normal skin control specimens from reduction plastic surgeries. Our pathologic cases were diagnosed by correlating clinical, epidemiologic, histopathologic, and immunologic methods; these methods included immunoblotting and enzyme-linked immunosorbent assay (ELISA) testing in selected cases. Our DIF was additionally performed as previously described.^[4] In brief, for DIF, we obtained the biopsies in Michel's transport medium at room temperature, and stored at 4°C until it was cut. Before cutting, we washed the biopsies in Michel's washing medium, and/or in phosphate-buffered saline (PBS) (pH 7.2) for 10-15 min. For frozen sections, the tissue was embedded in optimum cutting temperature (OCT) medium and sections of 4-5 micron thickness were cut. We used three sections on each slide, employing a pap pen to help avoid spreading one antibody into the other. We utilized fluorescein isothiocyanate (FITC)-conjugated

rabbit antisera to human immunoglobulin (Ig)G, IgA, IgM, Complement/C1q, Complement/C3, fibrinogen, and albumin. Specifically, FITC-conjugated rabbit antihuman IgG (1:25), IgA (1:25), and IgM (1:25) were used. For the antihuman fibrinogen and anti-albumin antibodies, we used 1:40 dilutions. The preceding antisera were purchased from Dako (Carpinteria, California, USA). In addition, we utilized FITC-conjugated goat antihuman IgE, (Kent Laboratories, Bellingham, Washington, USA) and antihuman FITC-conjugated IgD antibodies (Southern Biotechnology, Birmingham, Alabama). The slides were counterstained with 4', 6-diamidino-2-phenylindole (DAPI) (Pierce, Rockford, Illinois, USA), washed, coverslipped, and dried overnight at 4°C. Finally, we additionally used rhodamine-conjugated *Ulex Europaeus Agglutinin 1 (Ulex)* (Vector Laboratories, Burlingame, California, USA).

Statistical analysis

We stained 45 normal controls from plastic surgery reductions. We compared the controls with the pathologic skin conditions mentioned above. Two observers, each blinded to the sample identities, read both the DIF and the PAS stains.

Results

The 45 normal skin biopsies did not have any significant reactivity pattern. In 5/45 skin biopsies, some reinforcements were seen around eccrine sweat glands and their ducts. In 5/5 lupus cases, all of them were positive along the primary basement membrane zone (BMZ), as well as along eccrine gland BMZs, and sebaceous gland BMZs. The PAS findings were similar to those of the DIF [see Table 1]. Of interest, colloid bodies have been noted in lupus as well as LP cases by both methods. In regard to the samples with LP, 9/10 were positive by PAS in a linear pattern, and by DIF 10/10 were positive. In the cases of vasculitis, we noted positivity

Table 1: Comparison between PAS and DIF reactivity

| Diagnosis and number of cases | PAS | DIF | Positive area |
|---|------|-------|---|
| Skin lupus | 5/5 | 5/5 | Basement membrane zone. |
| Bullous pemphigoid | 5/5 | 5/5 | Basement membrane zone. |
| Pemphigus | 5/5 | 5/5 | Intercellular staining between keratinocytes |
| Vasculitis | 9/10 | 10/10 | Superficial and some intermediate/or deep skin vessels. |
| Drug eruptions, including erythema multiforme | 5/5 | 5/5 | Superficial skin vessels sweat glands |
| Lichen planus | 9/10 | 10/10 | Basement membrane zone |

to the superficial, the intermediate and/or deep vessels of the dermis; in each case, the level of the positivity in the vessels was the same with both techniques (with the exception of one case that was negative, or very weak positive by PAS and was positive by DIF [see Table 1]). In regard to the cases of erythema multiforme, we additionally found a correlation between the positivity and the anatomic level of the reactivity, with both PAS and DIF. The most severe cases diagnosed as erythema multiforme on H&E showed the strongest reactivity to the vessels; the majority were superficial, the intermediate depth vessels were second, and deep vessels the last. In the cases of severe drug reactions, the PAS failed to show staining; staining was noted mostly with FITC conjugated antifibrinogen on DIF, against multiple cell junctions in the epidermis and cell junctions of an unknown nature in the dermis. In the cases of pemphigus and pemphigoid, both techniques (PAS and DIF) showed similar results and staining patterns [see Table 1].

Table 1 shows a case-control study design was used to compare DIF positivity *versus* PAS stain positively. Our interrater reliability (agreement or concordance among the raters) was 95%. The score indicates how much consensus was found between the observers. The main problem we encountered in this context was that in a few cases, the PAS staining and the DIF staining were fainter than in other cases. Because the comparison

was between DIF and PAS staining, autoantibody titers were not reviewed. The positive PAS reactivity was determined as positive or negative. The positivity of the other dermatosis on DIF was read as positive or negativity as well.

In Figures 1-3, we show specific examples of the disease specimens we examined and the results of the H&E, PAS, and DIF staining.

Discussion

The detection of immunoglobulin or complement deposits has a diverse diagnostic value for different inflammatory and autoimmune dermatoses.^[2,3] While for autoimmune blistering diseases, the detection of tissue-bound autoantibodies are essential diagnostic criteria, revealing deposits of immunoreactants is helpful, but not strictly required for diagnosis of LE and LP^[2,3]

Antibodies and autoantibodies are immune system proteins called immunoglobulins, and they are rich in polysaccharides.^[7-9] In addition, autoimmune diseases often attack carbohydrate epitopes in the body. Immunoglobulins are primarily glycoprotein molecules produced by B lymphocytes and plasma

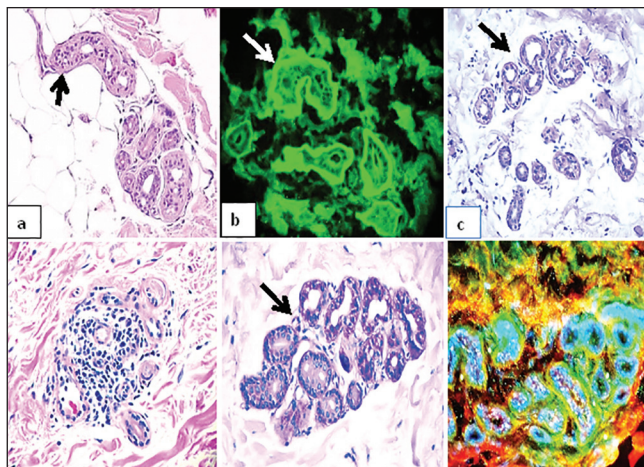


Figure 1: (a-c) A representative case of erythema multiforme. (a) H&E staining, showing edematous, large eccrine sweat glands (black arrow) (200×) (b) DIF, using FITC conjugated antifibrinogen; note positive staining around the same glands (green staining; white arrow) (c) PAS stain, showing mild positivity against the same glands (purple staining; black arrow). (d-f) A representative example of a drug eruption. (d) H&E staining showing edematous eccrine duct (black arrow) (400×) (e) PAS stain, showing positivity against a nearby eccrine gland (purple/red staining; black arrow) (f) DIF using FITC conjugated antihuman Complement/C3, and showing positive staining around the sweat glands (green staining). The nuclei of the cells were counterstained with DAPI in blue, and the blood vessels around the sweat gland stained with *Ulex* in red

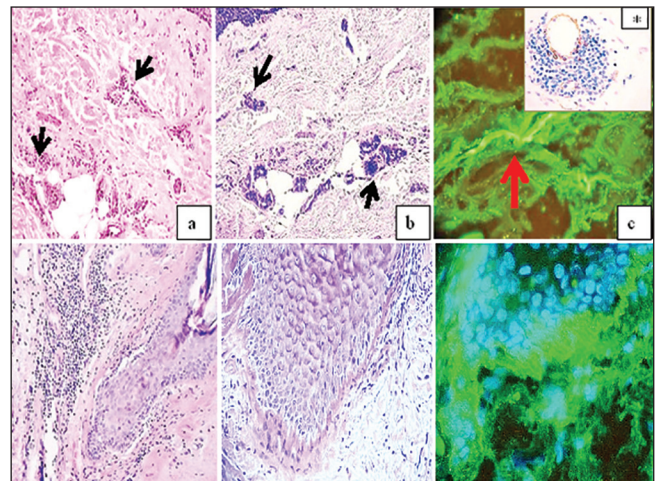


Figure 2: (a-c) A case of a vasculitis. (a) H&E staining, showing an upper dermal perivascular infiltrate (black arrows) (100×) (b) Positive staining with PAS around the same vessels as in a; the observed staining was not exclusively on neutrophils (purple staining; black arrows) (100×) (c) DIF, showing positive staining with fibrinogen FITC conjugated, against the same vessels (green staining; red arrow) (400×). The identity of the blood vessels was confirmed using CD31 immunohistochemistry. (d-f) A case of cutaneous lupus. (d) H&E staining shows damage to the basement membrane zone at the area of a hair follicle, and the inflammatory infiltrate (black arrow) (100×) (e) Shows a highlight of d, at the basement membrane zone (black arrow) (200×) (f) Shows DIF positive staining at the basement membrane zone of the sweat glands, using FITC conjugated anti-human IgM (green staining; white arrow). The nuclei of epidermal keratinocytes were counterstained with DAPI (light blue)

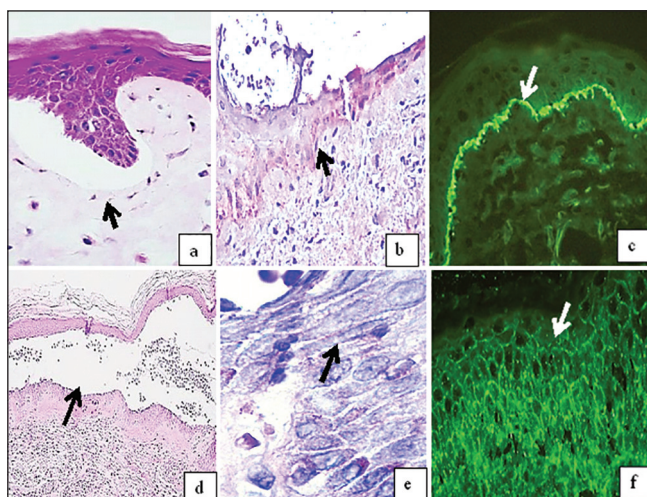


Figure 3: (a-c) A case of bullous pemphigoid. (a) Shows a subepidermal blister, with a few eosinophils near the base (black arrow) (200×) (b) Shows PAS positive staining at the basement membrane zone (light red staining; black arrow) (200×) (c) DIF positive staining, showing linear staining of FITC conjugated antihuman Complement/C3 at the basement membrane zone (green staining; white arrow). (d-f) A case of pemphigus vulgaris. (d) Classic intraepidermal blister (black arrow) (100×) (e) PAS staining, showing some intercellular staining between keratinocytes (purple staining; black arrow) (400×) (f) DIF, showing intercellular staining of IgG FITC conjugated in a “fish scale” pattern between epidermal keratinocytes (light green staining; white arrow) (200×)

cells in response to an immunogen. Our pilot study showed good sensitivity, specificity, and positive and negative predictive values when comparing both methods. We speculate that the correlation of staining positivity between PAS and DIF is due to the fact that reactive disease areas have stronger concentrations of polysaccharides, monosaccharides, and carbohydrates. Many reactive disease areas attract complement as well. Protein-carbohydrate interactions play critical roles in both the activation and regulation of complement factors.

Of interest, we were able to see the colloid bodies using both PAS and DIF. Colloid bodies are eosinophilic hyaline ovoid bodies that are often found in the subepidermal papillary regions or sometimes in the epidermis. They are usually seen in LP and LE. We speculate that these bodies may contain polysaccharides.

One problem observed in some samples was that the positive staining of PAS as well as DIF was very subtle. Thus, it was sometimes difficult to be certain as to which specific structures were exhibiting positive staining. Because our comparison was between DIF and PAS staining patterns, autoantibody titers were not studied.

A recent review^[10,11] postulated “The Altered Glycan Theory of Autoimmunity”; this theory suggests that each autoimmune disease may have an exclusive glycan

signature. The signature would be characterized by site-specific relative protrusions of individual glycan structures on immune cells and extracellular proteins, especially, site-specific glycosylation patterns of individual Ig classes and subclasses.^[10,11]

Further studies could record a scale of positivity of PAS (most pathologists simply report positive or negative staining), and additionally report autoantibody titers; these attempts are beyond the scope of our pilot study. Additional studies could additionally determine the positivity of PAS in other dermatoses, and compare these with DIF; these disorders could include autoimmune diseases in the oral mucosa; but we had no access to oral specimens for our study.

Conclusion

We conclude that laboratories lacking DIF, review a PAS slide in suspected cases of cutaneous autoimmune disorders. The PAS data, combined with a clinical history and H&E staining, may suggest an autoimmune skin disease diagnosis. Additionally, confirmatory DIF data would still be warranted, if clinically indicated.

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Conflicts of interest

There are no conflicts of interest.

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