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Enzyme-linked immunosorbent assay as a helpful diagnostic tool for pemphigus erythematosus with equivocal histologic and immunofluorescent findings

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Abstract

Enzyme-linked immunosorbent assay is a sensitive and specific method for the detection of circulating autoantibodies in pemphigus vulgaris and foliaceus. Herein, pemphigus erythematosus with equivocal immunofluorescence and non-diagnostic histology, but confirmed by enzyme-linked immunosorbent assay, is described. As a non-invasive, sensitive, and specific assay with additional utility for monitoring disease activity, this case adds to growing evidence supporting ELISA as the diagnostic method of choice for common and less common variants of pemphigus.

Keywords: pemphigus foliaceus, autoimmune blistering disorder, immunofluorescence, enzyme-linked immunosorbent assay

Introduction

Pemphigus foliaceus (PF) is an autoimmune disease characterized by superficial blisters. It is caused by immunoglobulin (Ig) G autoantibodies that target desmoglein (DSG) 1 and is the second most prevalent subtype of pemphigus [1, 2]. Immunofluorescence, histology, and more recently, enzyme-linked immunosorbent assay (ELISA) support the clinical diagnosis of pemphigus. Selecting the best test for diagnosis may pose a challenge. Direct immunofluorescence (DIF), in tandem with routine histology, is the most

commonly utilized diagnostic confirmatory method [3, 4]. However, recent evidence identifies ELISA as more accurate than DIF and histopathology for the diagnosis of pemphigus vulgaris (PV) and PF. Less evidence supports the utility of ELISA for the diagnosis of uncommon variants of pemphigus, such as pemphigus erythematosus (PE).

Case Synopsis

A 65-year-old woman with a history of rheumatoid arthritis presented for evaluation of a chronic blistering eruption of 6 months duration associated with pruritus. Multiple scattered ruptured bullae and erosions with crust and hyperpigmentation were present on her chest and upper back (Figure 1A). Mucosal involvement was absent. Histopathology failed to identify a discrete intraepidermal split, but eosinophilic spongiosis was demonstrated (Figure 1B). DIF of perilesional skin revealed granular IgM, IgG, C3, and C5b-9 deposition at the dermoepidermal junction (Figures 2A-2D, respectively). Weak, focal cell surface IgG reactivity (Figure 2B) was considered equivocal. Owing to the keratinocyte nuclear immunofluorescence (*in vivo* antinuclear antibody [ANA], Figure 2B), this lupus band was suggestive of autoimmune connective tissue disease (AICTD) such as systemic lupus erythematosus (SLE).

Given the association of eosinophilic spongiosis with clinical findings of an autoimmune bullous disorder, ELISAs for autoantibodies against bullous pemphigoid antigen (BPAG) 1 and 2 and DSG 1 and 3

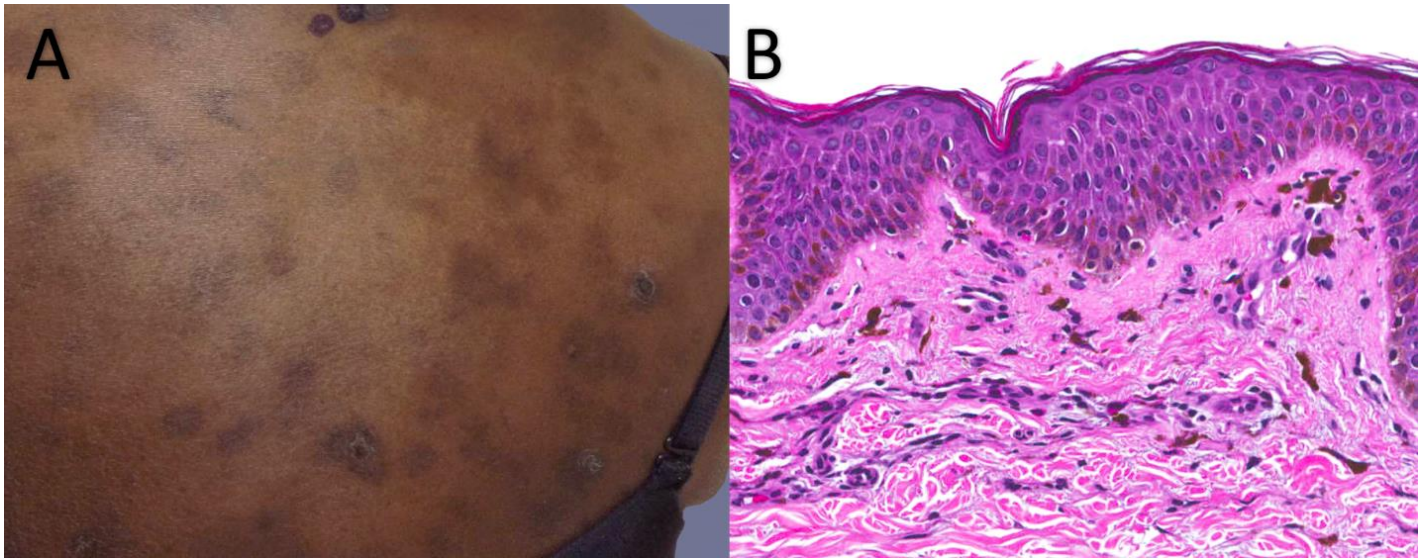


Figure 1. A) Healed superficial bullae, crusted erosions, and post-inflammatory hyperpigmentation on the upper back. B) Mild spongiosis with rare eosinophils present in the basilar epidermis. H&E, 200x.

were evaluated. Anti-DSG1 was 120U (reference range: negative <14, positive >20), diagnostic of PF; the other 3 serologies were negative. Rheumatoid factor level was 127 IU/mL and IgG for cyclic citrulline peptide IgG was measured at 166U. Treatment with class II topical corticosteroids over 6 weeks was

initially sufficient, but subsequent flaring required addition of mycophenolate mofetil, which produced remission.

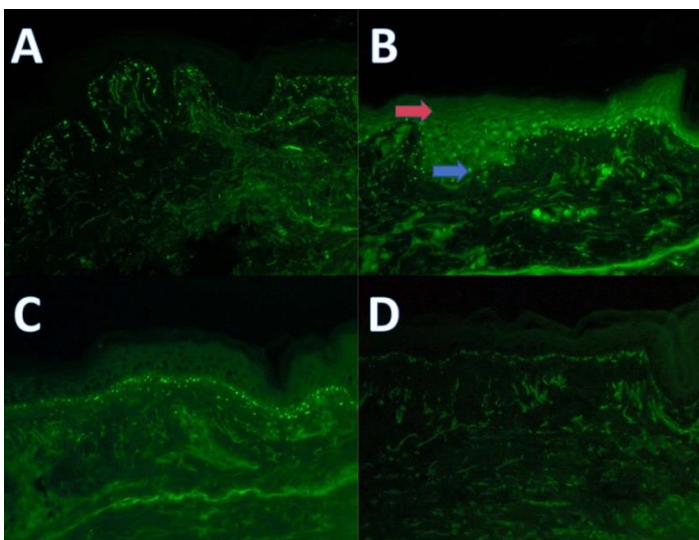


Figure 2. A) Granular IgM deposition at the dermoepidermal junction. Anti-IgM, 400x. B) Granular IgG deposition and the basement membrane along with in vivo antinuclear antibody, demonstrated by keratinocyte nuclear staining (blue arrow). Red arrow indicates weak focal (equivocal) cell surface IgG deposition. Anti-IgG, 400x. C) Prominent granular C3 deposition at the dermoepidermal junction (Anti-C3, 400x. D) C5b-9 junctional deposition was also identified. Anti-C5b-9, 400x.

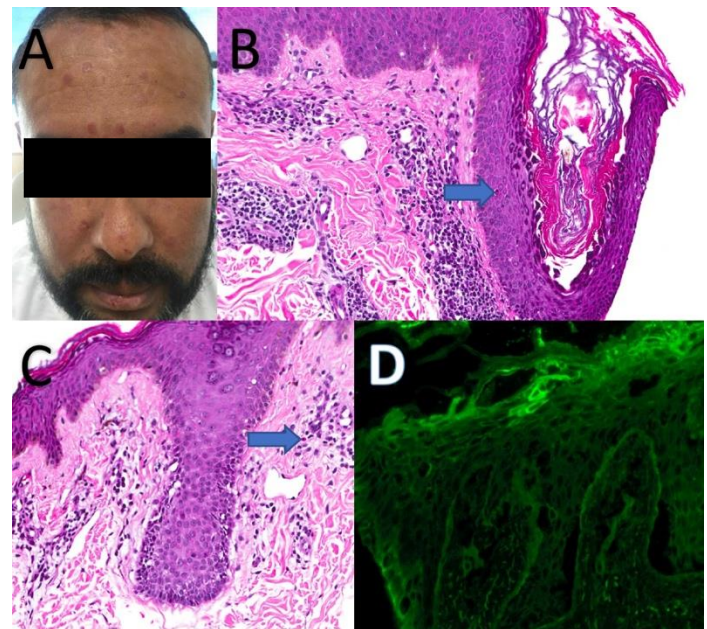


Figure 3. A) Localized, thin plaques with fine scale with a seborrheic distribution in a patient with localized pemphigus foliaceus. B) Prominent acantholysis involves the granular layer of the follicular infundibulum; rare dyskeratosis (arrow) is also present. H&E, 200x. C) Follicular spongiosis and acantholysis along with rare dermal eosinophils (arrow). H&E, 200x. d) Focal intercellular IgG deposition limited to the superficial (granular) epidermis, without junctional immunoreactant deposition. Anti-IgG, 400x.

Table 1. Sensitivity and specificity of DIF, IIF, and ELISA for the diagnosis of PV and PF.

	PV		PF	
	Sensitivity	Specificity	Sensitivity	Specificity
DIF [8, 9]	Approaches 100%	36%	Approaches 100%	36%
IIF [9]	81-95%	92%	81-95%	81-95%
ELISA [1]	97%	98%	96%	99%

DIF: direct immunofluorescence; IIF: indirect immunofluorescence; ELISA: enzyme-linked immunosorbent assay; PV: pemphigus vulgaris; PF: pemphigus foliaceus

Case Discussion

PE, also known as Senear-Usher syndrome, remains a controversial nosologic entity with variable criteria for diagnosis. When considered as a clinical subtype of PF, it has been described as a localized or early variant, with a predilection for the seborrheic and/or

malar regions (Figure 3). By contrast, classic PE, as an immunopathologic variant represented by the case presented herein (Figures 1 and 2), is characterized by overlapping features of SLE and PF, including deposition of multiple immunoreactants in a granular pattern (analogous to that seen in lupus band), keratinocyte nuclear fluorescence (*in vivo* ANA), and IgG on keratinocyte cell surfaces. *In vivo* ANA is also demonstrable in dermatomyositis, subacute cutaneous lupus erythematosus, and mixed connective tissue disease. Serologic evaluation may also demonstrate overlapping features of SLE, including ANA with significant titers in 40% of patients [5].

Recent studies have demonstrated that ELISA is a highly sensitive and specific method for diagnosing pemphigus. ELISA for anti-DSG1 boasts a sensitivity of 96% and a specificity of 99% [6]. The sensitivity

Table 2. Cases of PE with diagnostic support by ELISA [18,19,20].

Author	Age and gender	Clinical findings	Histology	DIF	ELISA	Associated diseases and laboratory findings
Gomi et al. [20]	30; F	Erosions localized to face	Superficial acantholysis	CS: IgG DEJ: IgG, IgM, C3	Anti-DSG1: 170	SLE
Karlhofer et al. [18]	43; F	Generalized erosions on the face, trunk, and extremities	Superficial acantholysis	CS: IgG, C3 DEJ: granular C3	Anti-DSG1: 199 Anti-BPAG1: 29	RA ANA 1:160 RF: 56.5 IU/mL
Karlhofer et al. [18]	22; F	Generalized erosions on the face, trunk, and extremities	Superficial acantholysis	CS: IgG, C3 DEJ: granular C3	Anti-DSG1: 177 Anti-BPAG1: 28	SLE ANA 1:320 Anti-dsDNA: 48.5 IU/mL
Karlhofer et al. [18]	34; F	Erosions localized to the chest	Superficial acantholysis	CS: IgG, C3 DEJ: granular C3	Anti-DSG1: 32 Anti-BPAG1: 16	
Akman et al. (n=5) [19]	43 (mean); 3 M, 2 F	NS	Superficial acantholysis	CS: IgG DEJ: linear C3, IgG	Anti-DSG1: 135 (mean)	ANA negative
Desai et al.	65; F	Superficial bullae localized to chest and back	Eosinophilic spongiosis without intraepidermal split	CS: weak or equivocal IgG DEJ: granular IgM, IgG, C3, C5b-9 <i>In vivo</i> ANA	Anti-DSG1: 120	RA ANA RF 127 IU/mL CCP: 166 U

PE: pemphigus erythematosus; ELISA: enzyme-linked immunosorbent assay; DIF: direct immunofluorescence; M: male; F: female; CS: cell surface; DEJ: dermoepidermal junction; IgG: immunoglobulin G; DSG: desmoglein; BPAG1: bullous pemphigoid antigen 1; RA: rheumatoid arthritis; ANA: antinuclear antibody (normal < 1:40); RF: rheumatoid factor (normal 0-15 IU/mL); SLE: systemic lupus erythematosus; dsDNA: double-stranded deoxyribonucleic acid (normal 0-7 IU/mL); NS: not specified; CCP: cyclic citrulline peptide IgG (normal 0-19 U)

and specificity of ELISA for anti-DSG3 in PV have been reported to be over 95% and 98%, respectively [7]. DIF approaches 100% sensitivity but cannot definitively distinguish PF from PV as both demonstrate bound intercellular IgG and complement [8, 9]. Thus, ELISA is almost as sensitive as, and more specific than, DIF for PF and PV.

By examining the level of acantholysis, one can often distinguish pemphigus vulgaris (PV) from PF. However, up to 25% of biopsies of PV and PF demonstrate variable levels of acantholysis, rendering routine histopathology insufficient for definitive diagnosis of common pemphigus subtypes [10]. In the case presented herein, the histopathology was even more nonspecific; eosinophilic spongiosis may be seen in pemphigus and pemphigoid group disorders, drug reactions, contact dermatitis, and incontinentia pigmenti [11,12,13]. Table 1 summarizes the sensitivity and specificity of DIF, IIF, and ELISA for the diagnosis of PV and PF.

In addition to its diagnostic utility, ELISA is quantitative and superior to DIF and indirect immunofluorescence (IIF) in assessing disease activity and treatment efficacy. Anti-DSG1 indices increase with the severity of PF and cutaneous PV whereas anti-DSG3 indices correlate with mucosal PV severity. By contrast, IIF does not correlate as strongly with disease activity at commonly observed titers [4]. As a caveat, at very high antibody levels, IIF is a more accurate quantitative marker than ELISA [14]. ELISA is also superior to DIF in determining serologic remission, with a sensitivity, specificity, positive predictive value, and negative predictive value of 100%, 80%, 61.1%, and 100%, respectively [15].

As an important caveat, DIF and ELISA are not sufficient in excluding paraneoplastic pemphigus (PNP), a disease defined by autoantibodies directed against the intracellular plakin family of proteins, with or without anti-DSG1 or -3 IgG. If PNP is a diagnostic consideration based on clinical findings or a lichenoid interface tissue reaction, the most

sensitive and specific diagnostic tests are immunoblotting for envoplakin and/or periplakin (sensitivity 100% and specificity 82-91%) and IIF on transitional epithelium (rat bladder, sensitivity 67-95%, specificity 100%), respectively [16, 17].

In contrast to more common variants of pemphigus, the utility of ELISA has been only infrequently described for PE [18-20]. Given the serologic and clinical overlap with AICTDs including SLE, and the reported co-presence of circulating autoantibodies against BPAG1 (230 kd) and periplakin (190 kd), [18], ELISA may serve as a highly accurate diagnostic method in cases of PE with otherwise confounding immunopathologic or serologic profiles. ELISA may also provide resolution in cases with additionally non-diagnostic histology, as in the case presented here. Table 2 summarizes the reported cases of PE in which ELISA demonstrated circulating autoantibodies directed against DSG1 [18-20]. Compared to these prior reports, the case described herein is unique given the high index of anti-DSG1 despite the absence of acantholysis and definitive cell surface IgG deposition. Without the diagnostic support provided by ELISA, the immunopathologic findings of granular immunoreactants at the dermoepidermal junction would have suggested AICTD.

Conclusion

ELISA for anti-DSG is a non-invasive and highly accurate test that demonstrates roughly equal sensitivity but greater specificity than histopathology and DIF for the diagnosis of PF and PV [10, 15]. ELISA should also be considered as a helpful diagnostic tool for PE in the context of noncorrelative, confounding, or non-diagnostic immunohistologic findings.

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