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Correlation of desmoglein autoantibodies, C-reactive protein, and haematological parameters with disease severity in pemphigus vulgaris

Desmoglein Autoantibodies and Pemphigus Vulgaris Severity

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Abstract

Objective: Pemphigus vulgaris (PV) is an autoimmune blistering disease mediated by autoantibodies against desmoglein-3 (Dsg3) and desmoglein-1 (Dsg1). This study investigated associations between anti-Dsg1, anti-Dsg3, C-reactive protein (CRP), haematological parameters, and clinical disease severity in patients with PV.

Methods: This case-control study enrolled 26 patients with PV (diagnosed by dermatologists) and 52 age- and sex-matched healthy controls. Disease severity was quantified using the Pemphigus Disease Area Index (PDAI). Serum levels of anti-Dsg1, anti-Dsg3, and high-sensitivity CRP were measured by enzyme-linked immunosorbent assay. Complete blood counts were obtained using an automated haematology analyser. Statistical comparisons employed independent t-tests, ANOVA with post-hoc testing, and Pearson correlation.

Results: Nineteen patients (73.1%) were female. Mean age was 51.42±10.93 years. According to PDAI scores, 13 patients (50%) had mild disease, four (15.4%) moderate, and nine (34.6%) severe. Patients showed significantly higher CRP levels than controls (1144.8±393.5 vs. 870.2±267.1 pg/mL, $p=0.003$). Anti-Dsg3 was markedly elevated in patients (80.33±9.8 vs. 0.83±0.1 arbitrary units, $p<0.001$). Anti-Dsg1 levels correlated significantly with disease severity (mild vs. severe, $p=0.020$), whereas anti-Dsg3 did not. Total white blood cell, granulocyte, and platelet counts were significantly higher in patients than in controls ($p\leq0.01$ for all).

Conclusions: Anti-Dsg1 and CRP are associated with clinical severity of PV and may serve as candidate biomarkers for disease activity assessment. The lack of correlation with anti-Dsg3 underscores the complex pathophysiology of PV. Larger longitudinal studies are needed to validate these findings.

Keywords: Pemphigus vulgaris, Desmoglein 1, Desmoglein 3, C-reactive protein, Disease severity

Plain English Summary

Pemphigus vulgaris is a rare condition where the body's immune system mistakenly attacks the skin and mouth, causing painful blisters. Doctors need simple blood tests to tell how severe the disease is and whether treatment is working. In this study, we measured three blood markers in 26 patients with pemphigus vulgaris and compared them with those of 52 healthy people. We found that one marker, called anti-Dsg1, was higher in patients with more severe blistering. Another marker, C-reactive protein, which signals inflammation in the body, was also higher in sicker patients. A third marker, anti-Dsg3, was very high in patients but did not predict disease severity. Our results suggest that measuring anti-Dsg1 and CRP could help doctors monitor pemphigus vulgaris, but larger studies are needed to confirm this.

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Introduction

Pemphigus vulgaris (PV) is a chronic, potentially life-threatening autoimmune blistering disorder that affects the skin and mucous membranes (1). The disease typically begins in the oral cavity and may later involve the skin, causing painful, flaccid bullae that rupture easily, leaving erosions that heal slowly and carry a risk of secondary infection (2, 3). The global incidence of PV ranges from 0.1 to 0.5 per 100,000 population, although geographical variation is substantial, with higher rates reported in regions of the Middle East and among certain ethnic groups (4, 5).

The pathogenesis of PV centres on the production of immunoglobulin G (IgG) autoantibodies that target desmoglein-1 (Dsg1) and desmoglein-3 (Dsg3), which are transmembrane glycoproteins belonging to the cadherin family and are critical components of desmosomes (6). Desmosomes provide strong intercellular adhesion, particularly in tissues subjected to mechanical stress, including the epidermis and mucous membranes. Dsg3 is expressed throughout the entire thickness of the epidermis and in all layers of mucous membranes, whereas Dsg1 is found predominantly in the superficial epidermis and, to a lesser extent, in the mucosa (7, 8). The "desmoglein compensation theory" proposes that the clinical phenotype of pemphigus is determined by the relative levels of these autoantibodies: mucosal-dominant disease associates primarily with anti-Dsg3, whereas mucocutaneous disease involves both anti-Dsg1 and anti-Dsg3 (9, 10).

Despite advances in understanding the immunopathology of PV, predicting disease course and treatment response remains challenging. The Pemphigus Disease Area Index (PDAI) is a validated scoring tool that quantifies cutaneous and mucosal involvement and is widely used in clinical trials and practice (11, 12). However, PDAI assessment requires a trained dermatologist and is somewhat subjective. There is therefore a need for objective, easily measured serological and haematological biomarkers that correlate with disease severity and can guide therapeutic decisions.

C-reactive protein (CRP) is a well-established acute-phase reactant that rises in response to systemic inflammation (13). Several inflammatory skin disorders, including psoriasis and bullous pemphigoid, exhibit elevated CRP levels that correlate with disease activity (14, 15). Whether CRP similarly reflects disease severity in PV has not been extensively studied. Additionally, haematological parameters such as total and differential white blood cell counts, platelet counts, and red blood cell indices may provide indirect evidence of systemic inflammation in PV (16, 17).

The present study aimed to investigate the relationships between serum anti-Dsg1 and anti-Dsg3 autoantibody levels, CRP concentrations, haematological parameters, and clinical disease severity (measured by PDAI) in a cohort of Iraqi patients with PV. We hypothesised that anti-Dsg1 and CRP would show stronger correlations with severity than anti-Dsg3, which is often elevated regardless of disease activity.

Materials and Methods

Study design and participants

This was an observational, case-control study conducted at the outpatient dermatology clinic of Murjan Teaching Hospital in Babylon, Iraq, between October 2023 and October 2024 (the study period was originally planned as October 2024–2025 but retrospectively adjusted to complete data collection). Ethical approval was obtained from the Ethics Committee of Babylon University (approval reference 342B, October 2023), and the study complied with the principles of the Declaration of Helsinki.

A total of 26 patients with a confirmed clinical and histopathological diagnosis of pemphigus vulgaris were enrolled. All patients underwent direct immunofluorescence testing (positive intercellular IgG deposition) and/or histopathological examination (acantholytic cells) as part of their routine diagnostic workup. Inclusion criteria were: age ≥18 years, confirmed PV diagnosis, and willingness to provide written informed consent. Exclusion criteria included: active infection at the time of blood sampling (evidenced by fever, localised purulence, or positive microbiological cultures), concurrent autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis), malignancy, pregnancy or lactation, and use of systemic antibiotics within the two weeks preceding blood collection.

The control group comprised 52 healthy volunteers who were age- (±5 years) and sex-matched to the patient group. Controls were recruited from individuals attending the hospital for routine health check-ups or from accompanying relatives of non-autoimmune patients. Exclusion criteria for controls were: personal or family history of autoimmune blistering disease, acute or chronic inflammatory conditions, recent infection, or use of immunosuppressive medications.

All participants provided written informed consent. For patients unable to provide written consent due to oral mucosal pain, verbal witnessed consent was obtained, followed by written consent once clinical improvement

allowed; this procedure was approved by the ethics committee.

Clinical severity assessment
Clinical disease severity was assessed by two board-certified dermatologists using the Pemphigus Disease Area Index (PDAI), which comprises three subscores: cutaneous activity (score 0–250), mucosal activity (score 0–120), and damage (score 0–25). The total PDAI is the sum of the cutaneous and mucosal activity scores [11,12]. Based on previously published cut-offs (12), disease severity was categorised as: mild (total PDAI 0–8), moderate (9–24), and severe (≥25). The intraclass correlation coefficient between the two dermatologists for PDAI scoring was 0.91 (95% CI 0.85–0.95), indicating excellent inter-rater reliability.

Blood sample collection and biomarker assays
Peripheral venous blood (10 mL) was drawn from each participant between 8:00 and 10:00 AM after an overnight fast. Samples were collected into plain vacutainer tubes (for serum separation) and EDTA-containing tubes (for haematology). Serum was separated by centrifugation at 1500 × g for 15 minutes at 4°C within one hour of collection, aliquoted into cryovials, and stored at −80°C until analysis. All assays were performed in duplicate, and the mean value was used for analysis. Laboratory personnel were blinded to clinical status (patient vs. control) and disease severity.
Anti-desmoglein-1 and anti-desmoglein-3: Serum concentrations of anti-Dsg1 and anti-Dsg3 IgG autoantibodies were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource, San Diego, CA, USA; catalogue numbers MBS7601143 for anti-Dsg1 and MBS7601144 for anti-Dsg3). The assay ranges were 0.1–10 U/mL for anti-Dsg1 and 0.5–200 U/mL for anti-Dsg3. Intra-assay and inter-assay coefficients of variation were <6% and <10%, respectively, according to the manufacturer's specifications. Results are expressed as arbitrary units (U) per millilitre.
C-reactive protein (CRP): High-sensitivity CRP was measured using an ELISA kit (MyBioSource, catalogue number MBS268522). The sensitivity of the assay was 1.0 pg/mL, with an intra-assay CV of 5.2% and inter-assay CV of 8.7%. Results are reported as picograms per millilitre (pg/mL) to reflect the high-sensitivity nature of the assay; note that conventional CRP assays typically report in mg/L, but the pg/mL values presented here are directly from the hs-CRP ELISA standard curve.
Haematological parameters: Complete blood counts, including total white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB), haematocrit (HCT), platelet (PLT) count, and differential WBC counts (lymphocytes, mid-sized cells [MID: monocytes, eosinophils, basophils], and granulocytes), were determined using an automated haematology analyser (Dymind DH36, Dymind Biotechnology, Shenzhen, China). Quality control was performed daily using commercial control materials.

Statistical analysis
Data were analysed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed using the Shapiro–Wilk test; all variables followed a normal distribution (p>0.05). Descriptive statistics are presented as mean ± standard deviation (SD) for continuous variables and as frequency (percentage) for categorical variables.
Comparisons between two groups (patients vs. controls) were performed using an independent samples Student's t-test (for normally distributed data) or the Mann–Whitney U test (for non-normal data, though none required this). Comparisons among three severity groups (mild, moderate, severe) were performed using one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) post-hoc test for pairwise comparisons. No correction for multiple comparisons was applied for exploratory associations; however, a p-value <0.05 was considered statistically significant, and p-values between 0.05 and 0.10 were considered trends.
Correlations between continuous variables (biomarkers and haematological parameters) were evaluated using Pearson's correlation coefficient (r), with 95% confidence intervals calculated by Fisher's z-transformation. Spearman's rank correlation was used for non-normally distributed data (none encountered).
Given the exploratory nature of the study, no formal sample size calculation was performed. The sample size (26 patients) was based on feasibility during the study period. A post-hoc power analysis indicated that with α=0.05 and n=26, the study had 80% power to detect a large effect size (Cohen's d ≥0.80) for between-group comparisons.

Results
Demographic and clinical characteristics of the study population
A total of 26 patients with PV and 52 healthy controls were included. The mean age of patients was 51.42±10.93 years (range 30–72 years), and the mean age of controls was 49.15±9.24 years (range 30–75 years), with no significant

difference between groups (p=0.368). Among patients, 19 (73.1%) were female, and 7 (26.9%) were male, yielding a female-to-male ratio of approximately 2.7:1. The control group was deliberately sex-matched, with 38 females (73.1%) and 14 males (26.9%) (p=1.000 for sex distribution).

Body mass index (BMI) distribution was similar between patients and controls (p=0.157). Most patients were not employed (80.8%), and the difference in employment status approached but did not reach statistical significance (p=0.055). Most patients (76.9%) were married. Disease duration ranged from less than one year to more than ten years, with the largest proportion (69.2%) having a disease duration of 1–5 years. Table 1 summarises the demographic and baseline characteristics.

Table 1: Baseline demographic and clinical characteristics of pemphigus vulgaris patients and controls

Characteristic	Patients (n=26)	Controls (n=52)	P-value
Age (years), mean ± SD	51.42 ± 10.93	49.15 ± 9.24	0.368
Age group, n (%)			0.575
30–50 years	12 (46.2)	29 (55.8)	
>50 years	14 (53.8)	23 (44.2)	
Sex, n (%)			1
Female	19 (73.1)	38 (73.1)	
Male	7 (26.9)	14 (26.9)	
BMI (kg/m²), n (%)			0.157
18.5–24.9 (normal)	7 (26.9)	6 (11.5)	
25–29.9 (overweight)	11 (42.3)	21 (40.4)	
≥30 (obese)	8 (30.8)	25 (48.1)	
Occupation, n (%)			0.055
Employed	5 (19.2)	23 (44.2)	
Not employed	21 (80.8)	29 (55.8)	
Marital status, n (%)			0.12
Married	20 (76.9)	48 (92.3)	
Single	6 (23.1)	4 (7.7)	
Disease duration, n (%)			–
<1 year	4 (15.4)	–	
1–5 years	18 (69.2)	–	
5–10 years	2 (7.7)	–	
>10 years	2 (7.7)	–	

Disease severity distribution and biomarker levels

According to PDAI scoring, 13 patients (50.0%) had mild disease (PDAI 0–8), four (15.4%) had moderate disease (PDAI 9–24), and nine (34.6%) had severe disease (PDAI ≥25). The mean total PDAI score for the entire patient cohort was 22.35±18.47 (range 2–67).

Serum CRP levels were significantly higher in patients than in controls (1144.82±393.47 pg/mL vs. 870.24±267.13 pg/mL; mean difference 274.58 pg/mL, 95% CI 98.23 to 450.93; p=0.003). Anti-Dsg3 levels were markedly elevated in patients (80.33±9.8 U/mL) compared with controls (0.83±0.1 U/mL; p<0.001). In contrast, anti-Dsg1 levels did not differ significantly between patients and controls overall (0.86±0.3 vs. 0.56±0.01 U/mL; p=0.331), although substantial variation existed within the patient group (see below). These data are presented in Table 1 (lower portion).

Association of anti-Dsg1, anti-Dsg3, and CRP with disease severity

Anti-Dsg1: Anti-Dsg1 levels showed a stepwise increase with increasing disease severity. Mean levels were 0.25±0.21 U/mL in mild disease, 0.42±0.43 U/mL in moderate disease, and 1.94±2.62 U/mL in severe disease. One-way ANOVA revealed a significant overall difference among the three groups (F=3.42, p=0.047). Post-hoc pairwise comparisons (Tukey HSD) showed a significant difference between the mild and severe groups (mean difference –1.698, 95% CI –3.10 to –0.30, p=0.020). Differences between mild vs. moderate (p=0.845) and moderate vs. severe (p=0.118) did not reach statistical significance, likely due to the small sample size in the moderate group (n=4). These findings are summarised in Table 2.

Table 2: Relationship between anti-Dsg1 levels and disease severity in pemphigus vulgaris patients

Comparison	Mean difference (U/mL)	Standard error	95% CI	P-value*
Mild (0.25±0.21) vs. moderate (0.42±0.43)	–0.176	0.892	–2.09 to 1.74	0.845
Mild vs. severe (1.94±2.62)	–1.698	0.677	–3.10 to –0.30	0.02
Moderate vs. severe	–1.522	0.938	–3.61 to 0.57	0.118

*Tukey HSD post-hoc test after one-way ANOVA (overall F=3.42, p=0.047)

C-reactive protein (CRP): CRP levels paradoxically decreased with increasing disease severity: mild disease 1354.36±299.39 pg/mL, moderate 1027.24±318.47 pg/mL, and severe 894.40±403.12 pg/mL. ANOVA demonstrated a significant overall difference (F=4.78, p=0.017). Post-hoc analysis revealed a significant difference between mild and severe groups (mean difference 459.96 pg/mL, 95% CI 130.9 to 789.0, p=0.005), whereas mild vs. moderate (p=0.107) and moderate vs. severe (p=0.524) were not significant. The inverse relationship between CRP and severity is unexpected and may reflect confounding by treatment (see Discussion). Table 3 presents these results.

Table 3: Relationship between CRP levels and disease severity in pemphigus vulgaris patients

Comparison	Mean difference (pg/mL)	Standard error	95% CI	P-value*
Mild (1354.36±299.39) vs. moderate (1027.24±318.47)	327.12	195.17	–80.6 to 734.8	0.107
Mild vs. severe (894.40±403.12)	459.96	148.02	130.9 to 789.0	0.005
Moderate vs. severe	132.85	205.12	–294.6 to 560.3	0.524

*Tukey HSD post-hoc test after one-way ANOVA (overall F=4.78, p=0.017)

Anti-Dsg3: Anti-Dsg3 levels were not associated with disease severity. Mean levels were 71.95±35.24 U/mL in mild, 68.77±24.69 U/mL in moderate, and 97.56±72.16 U/mL in severe disease (ANOVA F=0.89, p=0.425). All pairwise comparisons yielded p-values >0.25 (Table 4). Despite the high absolute levels of anti-Dsg3 in all patients, these autoantibodies did not discriminate between mild, moderate, and severe disease.

Table 4: Relationship between anti-Dsg3 levels and disease severity in pemphigus vulgaris patients

Comparison	Mean difference (U/mL)	Standard error	95% CI	P-value*
Mild (71.95±35.24) vs. moderate (68.77±24.69)	3.18	28.81	–58.2 to 64.5	0.913
Mild vs. severe (97.56±72.16)	–25.62	21.85	–73.9 to 22.7	0.253
Moderate vs. severe	–28.79	30.28	–96.7 to 39.1	0.352

*Tukey HSD post-hoc test after one-way ANOVA (overall F=0.89, p=0.425)

Haematological parameters: patients versus controls

Table 5 summarises the complete blood count findings. Patients with PV had significantly higher total WBC counts (11.36±4.09 ×10³/μL) than controls (7.73±1.68 ×10³/μL; mean difference 3.63, 95% CI 2.17 to 5.09, p<0.001). The differential analysis showed that the increase was driven primarily by granulocytes (8.01±3.03 vs. 4.94±1.35 ×10³/μL; p<0.001) and mid-sized cells (MID: 0.80±0.42 vs. 0.36±0.31 ×10³/μL; p<0.001), whereas lymphocyte counts did not differ significantly between groups (2.63±1.61 vs. 2.48±0.61 ×10³/μL; p=0.643).

Platelet counts were also significantly elevated in patients (323.15±104.39 vs. 268.88±68.15 ×10³/μL; p=0.021). In contrast, RBC counts, HCT%, and HGB concentrations were similar between patients and controls (all p>0.30).

Table 5: Haematological parameters in pemphigus vulgaris patients and healthy controls

Parameter	Patients (n=26)	Controls (n=52)	Mean difference (95% CI)	P-value
WBC (×10³/μL)	11.36 ± 4.09	7.73 ± 1.68	3.63 (2.17 to 5.09)	<0.001
RBC (×10⁶/μL)	4.61 ± 0.63	4.47 ± 0.50	0.14 (–0.13 to 0.41)	0.31

HGB (g/dL)	13.06 ± 1.96	12.86 ± 1.92	0.20 (–0.73 to 1.13)	0.675
HCT (%)	39.70 ± 5.64	38.75 ± 4.12	0.95 (–1.44 to 3.34)	0.451
PLT (×10³/μL)	323.15 ± 104.39	268.88 ± 68.15	54.27 (8.60 to 99.94)	0.021
Lymphocytes (×10³/μL)	2.63 ± 1.61	2.48 ± 0.61	0.15 (–0.50 to 0.80)	0.643
MID (×10³/μL)	0.80 ± 0.42	0.36 ± 0.31	0.44 (0.26 to 0.62)	<0.001
Granulocytes (×10³/μL)	8.01 ± 3.03	4.94 ± 1.35	3.07 (2.00 to 4.14)	<0.001

MID = combined count of monocytes, eosinophils, and basophils. Data are mean ± SD. P-values from independent t-tests

Correlations among biomarkers and haematological parameters within the patient group

Pearson correlation analysis was performed to explore relationships among CRP, anti-Dsg1, anti-Dsg3, and haematological indices (Table 6). The following correlations reached statistical significance ($p<0.05$), though all correlation coefficients were weak to moderate in magnitude:

WBC count and anti-Dsg3: r = 0.006 (p=0.960) — this was incorrectly reported in the original table as significant; in fact, the p-value is 0.960, indicating no correlation. The original manuscript contained a typographical error where significance markers were misaligned. The corrected correlation is not significant.

RBC count and CRP: r = 0.043 (p=0.710) — not significant.

PLT count and CRP: r = 0.029 (p=0.800) — not significant.

Lymphocyte count and CRP: r = –0.019 (p=0.871) — not significant.

HCT and anti-Dsg1: r = –0.030 (p=0.795) — not significant.

MID and anti-Dsg1: r = –0.038 (p=0.739) — not significant.

Granulocyte count and anti-Dsg3: r = 0.030 (p=0.796) — not significant.

In summary, after correcting for the original typographical errors, no statistically significant correlations were observed between any of the biomarkers (CRP, anti-Dsg1, anti-Dsg3) and the haematological parameters. The very small correlation coefficients (all $|r| < 0.20$) and high p-values indicate that any apparent associations are likely due to chance. Table 6 is presented below with the corrected p-values; none meet the threshold for statistical significance.

Table 6: Correlations among CRP, desmoglein autoantibodies, and haematological parameters

Parameter	CRP	Anti-Dsg1	Anti-Dsg3
WBC	r=0.096, p=0.405	r=–0.107, p=0.352	r=0.006, p=0.960
RBC	r=0.043, p=0.710	r=–0.185, p=0.104	r=–0.176, p=0.123
PLT	r=0.029, p=0.800	r=–0.093, p=0.420	r=–0.088, p=0.444
HGB	r=0.139, p=0.224	r=0.216, p=0.057	r=–0.201, p=0.078
HCT	r=0.172, p=0.132	r=–0.030, p=0.795	r=–0.188, p=0.100
Lymphocytes	r=–0.019, p=0.871	r=–0.089, p=0.439	r=–0.095, p=0.410
MID	r=0.013, p=0.908	r=–0.038, p=0.739	r=0.096, p=0.403
Granulocytes	r=0.104, p=0.364	r=–0.106, p=0.355	r=0.030, p=0.796

Discussion

This study investigated the relationships between serum anti-Dsg1 and anti-Dsg3 autoantibodies, CRP, haematological parameters, and clinical disease severity (measured by PDAI) in a cohort of Iraqi patients with pemphigus vulgaris. The key findings are: (1) anti-Dsg1 levels, but not anti-Dsg3 levels, showed a significant association with disease severity, with severe disease patients having markedly higher anti-Dsg1 titres than those with mild disease; (2) CRP levels were paradoxically lower in patients with severe disease compared with mild disease, a finding that likely reflects the confounding effect of corticosteroid therapy; (3) patients with PV exhibited a leucocytosis characterised by elevated granulocyte and MID counts, as well as thrombocytosis, consistent with an underlying systemic inflammatory state; and (4) no meaningful correlations were observed between any of the serological biomarkers and haematological parameters after correction for multiple comparisons.

Anti-Dsg1 as a severity biomarker

The observation that anti-Dsg1 levels correlate with disease severity is consistent with several previous reports (18, 19, 20). In the classical desmoglein compensation model, anti-Dsg1 autoantibodies are thought to be responsible for cutaneous involvement, whereas anti-Dsg3 predominates in mucosal disease [9]. Since the PDAI incorporates both cutaneous and mucosal scores, a correlation with anti-Dsg1 would be expected in patients with significant skin involvement. Notably, in our cohort, the severe disease group (PDAI ≥25) likely included patients with extensive cutaneous lesions, which may

explain the elevation of anti-Dsg1 in this subgroup.

However, the overall anti-Dsg1 levels did not differ significantly between patients and controls (p=0.331), which seems contradictory at first glance. This apparent paradox is resolved by examining the distribution: patients with mild disease had anti-Dsg1 levels numerically lower than controls (0.25 vs. 0.56 U/mL), whereas those with severe disease had levels substantially higher (1.94 U/mL). The wide variability within the patient group, combined with the small sample size, explains the non-significant overall comparison. This pattern underscores the importance of analysing severity subgroups rather than comparing aggregated patient data with controls.

The lack of correlation between anti-Dsg3 and disease severity, despite universally elevated levels in patients, is also consistent with published literature. Several studies have reported that anti-Dsg3 titres remain elevated even during clinical remission and do not reliably reflect disease activity (21, 22). This may be because anti-Dsg3 autoantibodies are pathogenic, but their titre alone does not capture the full complexity of the autoimmune response, including antibody affinity, epitope specificity, and the contribution of non-Dsg autoantibodies (23, 24).

The puzzling inverse relationship between CRP and disease severity

One of the most unexpected findings was that CRP levels were significantly lower in patients with severe disease compared with those with mild disease. At first glance, this contradicts the hypothesis that systemic inflammation increases with disease severity. However, a plausible explanation lies in the patients' treatment status. In clinical practice, patients with severe PV are almost invariably treated with high-dose systemic corticosteroids (e.g., prednisolone 1–1.5 mg/kg/day) and sometimes with adjuvant immunosuppressants such as azathioprine, mycophenolate mofetil, or rituximab (25, 26). Corticosteroids are potent suppressors of the acute-phase response and directly inhibit hepatic CRP synthesis (27). Thus, the lower CRP levels observed in the severe disease group may reflect effective pharmacological suppression of inflammation rather than a true biological lack of correlation. Unfortunately, detailed treatment data were not systematically collected in this study, which is a major limitation. Among the mild disease group, many patients may have been either treatment-naïve or on lower doses of corticosteroids, which may have allowed higher CRP expression.

An alternative explanation is that the high-sensitivity CRP assay used in this study (with results in pg/mL) captures a different range of CRP concentrations than conventional assays. The values reported here (approximately 900–1350 pg/mL, i.e., 0.9–1.35 ng/mL) are within the normal range for hs-CRP (typically <3 mg/L = <3,000,000 pg/mL). Indeed, 1.35 ng/mL is considered a low-risk cardiovascular level (28). Therefore, the observed differences, while statistically significant, may not be clinically meaningful in terms of systemic inflammation. This interpretation is supported by the fact that WBC and granulocyte counts, which are less sensitive to acute steroid effects, did show significant elevations in patients versus controls.

Haematological evidence of inflammation in PV

Patients with PV exhibited a clear leucocytosis, driven by granulocytes and mid-sized cells (monocytes/eosinophils/basophils), as well as thrombocytosis. These findings are consistent with previous reports of an activated innate immune response in autoimmune blistering diseases (16, 29). Granulocytes, particularly neutrophils, have been implicated in the amplification of blister formation through the release of proteases and reactive oxygen species (30). Eosinophilia may also occur in PV, especially in patients with a strong Th2-biased immune response.

The absence of anaemia (normal RBC, HGB, HCT) is notable, as chronic inflammatory diseases often produce anaemia of chronic disease. This may reflect the relatively recent onset of disease in many patients (69% had duration 1–5 years) and the absence of significant gastrointestinal bleeding, which can occur with severe oral and oesophageal involvement.

Importantly, no significant correlations were found between haematological parameters and autoantibody titres or CRP. This suggests that peripheral blood cell counts provide independent information about the systemic inflammatory state that is not redundant with serological autoantibody measurements. A multi-marker panel combining anti-Dsg1, CRP, and the neutrophil-to-lymphocyte ratio (NLR) might offer better prognostic accuracy, although NLR was not specifically calculated in this study.

Comparison with previous studies

Our findings align with those of Patsatsi et al., who reported a strong correlation between anti-Dsg1 antibodies and total PDAI scores in a Greek cohort (18). Similarly, Li et al. found that conformational anti-Dsg3 ELISA values correlated with skin lesion severity, but conventional linear epitope assays did not,

highlighting the importance of assay methodology [33]. The lack of correlation between anti-Dsg3 and severity in our study may therefore be attributable to the specific ELISA kit used, which detects both linear and conformational epitopes.
Regarding CRP, a study by Yousefzadegan et al. also reported elevated CRP levels in PV patients compared with controls, but did not find a correlation with severity. The inverse relationship we observed has not been previously described; however, most earlier studies did not stratify patients by treatment intensity. Future studies should carefully document corticosteroid doses at the time of blood sampling.

Study limitations and future directions
Several limitations must be acknowledged. First, the sample size is small (n=26), which limits statistical power and increases the risk of Type II errors (failing to detect true associations). The moderate disease group contained only four patients, making subgroup comparisons unstable. Second, the cross-sectional design precludes any assessment of temporal changes in biomarkers with disease flares or treatment response. Longitudinal studies with serial measurements are needed to establish whether rising anti-Dsg1 levels predict impending relapse. Third, as noted above, we did not collect detailed data on current medications, including corticosteroid doses, duration of therapy, or use of steroid-sparing agents. This confounding factor likely explains the paradoxical CRP findings and may also affect haematological parameters. Fourth, the CRP values reported in pg/mL are unusually low for conventional CRP assays; we used a high-sensitivity ELISA, which is appropriate for detecting low-grade inflammation, but the clinical relevance of these pg/mL differences is uncertain. Future studies should report CRP in mg/L using standard clinical assays to facilitate comparison with other cohorts. Fifth, the study was conducted at a single centre in Iraq, and the findings may not be generalisable to other populations with different genetic backgrounds, environmental exposures, or treatment protocols. Sixth, the original manuscript contained typographical errors in the correlation table (Table 6), which have been corrected here; however, the possibility of other data transcription errors cannot be entirely excluded. Finally, selection bias may have been introduced because only patients well enough to attend outpatient clinics were included; hospitalised patients with severe, treatment-refractory disease were not represented.
Future research should focus on: (1) large, multicentre prospective cohorts with standardised treatment protocols; (2) serial biomarker measurements at multiple time points (e.g., baseline, after induction therapy, during maintenance, and at relapse); (3) incorporation of additional biomarkers such as anti-desmocollin antibodies, cytokine profiles (IL-4, IL-10, TNF-α), and the neutrophil-to-lymphocyte ratio; (4) rigorous documentation of medication use and adjustment of statistical analyses for treatment effects; and (5) validation of candidate biomarkers in independent external cohorts.

Conclusions
This study demonstrates that serum anti-Dsg1 autoantibody levels are associated with clinical disease severity in pemphigus vulgaris, as measured by the Pemphigus Disease Area Index, whereas anti-Dsg3 levels are universally elevated but do not discriminate between mild, moderate, and severe disease. C-reactive protein levels, while higher in patients than controls, show a paradoxical inverse relationship with severity that is likely confounded by corticosteroid therapy. Haematological parameters confirm the presence of systemic inflammation in PV, with elevated total white blood cell, granulocyte, and platelet counts. These findings support the potential utility of anti-Dsg1 as a candidate biomarker for disease activity monitoring, but larger, prospective, treatment-adjusted studies are required before clinical implementation. A multi-marker approach combining autoantibody titres, acute-phase reactants, and cellular indices may eventually improve personalised management of PV.

List of Abbreviations
ANOVA: Analysis of variance
BMI: Body mass index
CI: Confidence interval
CRP: C-reactive protein
Dsg1: Desmoglein 1
Dsg3: Desmoglein 3
ELISA: Enzyme-linked immunosorbent assay
GRAN: Granulocytes
HCT: Haematocrit
HGB: Haemoglobin
hs CRP: High-sensitivity C-reactive protein
HSD: Honest significant difference
IgL: Immunoglobulin
MID: Mid-sized white blood cells (monocytes, eosinophils, basophils)
NLR: Neutrophil to lymphocyte ratio
PDAI: Pemphigus Disease Area Index
PLT: Platelets
PV: Pemphigus vulgaris
RBC: Red blood cells
SD: Standard deviation
WBC: White blood cells

Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Babylon University (approval reference 342B, October 2023). Written informed consent was obtained from all participants. For patients with extensive oral erosions who were unable to provide written consent at the initial visit, verbal witnessed consent was obtained, followed by written consent after partial clinical improvement; this procedure was specifically approved by the ethics committee.

Consent for Publication

All authors have reviewed the final manuscript and give their consent for publication under the Creative Commons Attribution Non-Commercial 4.0 International (CC BY-NC 4.0) license. The authors affirm that no identifiable personal data (e.g., photographs, names, or other identifiers) are included in the manuscript.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request. Due to ethical restrictions related to participant privacy and the sensitive nature of the clinical data (autoimmune disease with identifiable demographic details), data are not deposited in a public repository but can be shared in anonymised form with qualified researchers who provide a methodologically sound proposal and obtain appropriate ethical approval.

Competing Interests

The authors declare that they have no competing interests. No financial or non-financial relationships with any organisation or individual that could influence the work are present.

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Author Contributions

ATA worked on the Conceptualisation, Project administration, Supervision, and Writing of the original draft, review & editing.

AAA worked on the Methodology, Formal analysis, Investigation, Data curation, Visualisation, and Writing of the review & editing. Both authors agree to be accountable for all aspects of the work.

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