ORIGINAL ARTICLE

Skin Eosinophil Counts in Bullous Pemphigoid as a Prognostic Factor for Disease Severity and Treatment Response

Sharon BAUM[1,2#], Maya ENGLER MARKOWITZ[2#], Anna LYAKHOVITSKY[1,2], Rotem GERSHON[2], Hannah AVIV[1], Zvi SEGAL[1,2] and Aviv BARZILAI[1–3]

[1]Department of Dermatology and [3]Institute of Pathology, Sheba Medical Center, Tel HaShomer, and [2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

[#]These authors contributed equeally to this publication.

Dermal infiltration of eosinophils and eosinophilic spongiosis are prominent features of bullous pemphigoid lesions. Although several observations support the pathogenic role of eosinophils in bullous pemphigoid, few studies have examined the impact of skin eosinophil counts on disease severity and treatment response. This retrospective study assessed the association between eosinophil counts in skin biopsy samples of 137 patients with bullous pemphigoid and their demographic characteristics, comorbidities, disease severity, and treatment response. There was no relationship between eosinophil count and age, sex, or disease severity at disease onset. There was a positive relationship between eosinophil counts and neurological comorbidity and a negative relationship between eosinophil counts and treatment response. At all follow-up points patients with no tissue eosinophils had a better response to treatment than patients with any tissue eosinophil count. In conclusion, skin eosinophil counts in patients with bullous pemphigoid are not correlated with disease severity at onset, but can serve as a negative prognostic marker for treatment response.

Key words: bullous pemphigoid; tissue eosinophils; disease severity; treatment response.

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Corr: Sharon Baum, Department of Dermatology, Sheba Medical Center, Tel HaShomer, Ramat Gan, Israel. E-mail: sharon.baum@sheba.health.gov.il

SIGNIFICANCE

Bullous pemphigoid is an autoimmune blistering disease characterized by dermal infiltration of eosinophils and eosinophilic spongiosis. This study investigated the association between eosinophil counts in skin biopsy samples of 137 patients with bullous pemphigoid and their demographic characteristics, comorbidities, disease severity and treatment response. There was no relationship between eosinophil count and disease severity at disease onset. There was a positive relationship between eosinophil counts and neurological comorbidity and a negative relationship between eosinophil counts and treatment response. Patients with no tissue eosinophils had a better response to treatment than patients with any tissue eosinophil count. Therefore, skin eosinophil counts in patients with bullous pemphigoid are not correlated with disease severity at onset, but can serve as a negative prognostic marker for treatment response.

Bullous pemphigoid (BP) is the most common subepidermal autoimmune blistering disease. BP typically occurs in elderly individuals. The overall incidence of BP ranges from 2.4 to 21.7 new cases per 1 million individuals per year in different population across the world, and shows a trend of increase incidence within the years. It occurs equally in both sexes (1), and is associated with a high risk of mortality (2). The comorbidities most frequently described in association with BP include neurological disorders, which are observed in 28–56% of patients (2). BP has been associated with the use of certain drugs, such as dipeptidyl peptidase-4 inhibitors (DPP-4i). Clinically, patients most commonly present with blisters. Before the development of blisters, pruritus often occurs with or without urticarial lesions. Oral involvement is observed in 10–30% of patients (3). BP has also been diagnosed in patients presenting with isolated pruritus without skin lesions (2). However, the exact factors underlying the various clinical presentations of BP remain unclear.

The pathogenesis of BP is characterized by immunological and inflammatory processes that cause subepidermal splitting, leading to the formation of bullae. IgG autoantibodies against the hemi-desmosomal proteins BP180 and BP230 are detected in the skin and serum of patients with BP. Direct immunofluorescence (DIF) reveals linear deposition of IgG and C3 at the dermal-epidermal junction (DEJ) (4). Another key histological feature of BP is eosinophilic dermal infiltrate. Eosinophils are often scattered throughout the upper dermis or aggregated at the edge of the DEJ, and they are thought to have an active role in the pathogenesis of BP (5). Peripheral eosinophilia is present in up to 50% of patients, and studies have reported that their levels may be associated with disease activity (6–10).

Increased serum concentrations of secretory granules, such as eosinophil cationic protein (ECP), have been found in patients with BP; furthermore, their levels have been observed to correlate with disease severity (11). A similar relationship has been observed with interleukin

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(IL)-5 (eosinophil colony-stimulating factor) (12, 13). However, a previous study evaluating mepolizumab (IL-5 antagonist) in patients with BP did not show a positive effect on disease course (14). Although under this treatment there was a significant decrease in blood eosinophil count, there was no decrease in the relapse incidence. Therefore, a possible explanation for treatment failure is that the number of eosinophils infiltrating the skin has a greater effect on disease severity than the number of eosinophils in the blood (14). Data regarding the relationships between tissue eosinophil levels and disease severity and treatment outcomes are lacking.

ECP, IL-5, and several other biomarkers of eosinophils (IL-16, IL-17A, and IL-23) that are correlated with BP severity, are not available for common clinical practice; therefore, they are not used routinely (10, 15–17). Establishing the relationship between tissue eosinophils and treatment response may serve as a tool for clinicians to predict disease course, which could affect treatment choice. The aim of this study was to assess the association between cutaneous eosinophil counts in patients with BP and the patients' demographic characteristics and comorbidities, and to investigate the impact on disease severity and treatment outcomes.

MATERIALS AND METHODS

This was a retrospective study of patients diagnosed and treated at the dermatology department and outpatient clinic of the Sheba Medical Center, Ramat Gan, Israel, between January 2012 and February 2020. Data were collected using a computerized Chameleon software of Sheba Medical Center. The study was approved by the local ethics committee (SMC-7172-09). Inclusion criteria were: a confirmed diagnosis of BP based on the typical clinical picture and typical histological appearance of subepidermal splitting, with positive DIF results confirmed in Sheba Medical Center's laboratory or in a certified laboratory at another medical centre; hospitalized and/or followed-up for at least 1 month; underwent a skin biopsy at Sheba Medical Center; and not using systemic steroid treatment at the time the biopsy was performed. Only those patients who met all the inclusion criteria were included in the study.

Exclusion criteria were: an inconclusive diagnosis of BP, clinically, histology, or with a negative DIF study; follow-up of less than 1 month; and the biopsy was not performed at Sheba Medical Center, or the biopsy sample could not be evaluated for technical reasons.

Medical records were assessed for demographic characteristics, background diseases, and clinical presentation. Due to the retrospective nature of the study, the BP Disease Area Index (BPDAI) score was not available, because it was not part of the routine clinical follow-up. Instead, the body surface area (BSA) score was used, which was reported in the medical records during the research period. The skin lesion type (pruritus only, urticarial lesion, bullae), mucosal involvement (yes or no), laboratory test results (blood eosinophils), histopathological picture, DIF results, treatment type, and response to treatment were also evaluated.

All patients were treated with the same protocol according to their disease activity (18). Those with localized/limited disease with mild activity received first-line treatment including topical corticosteroids and second-line treatment including oral corticosteroids (0.3–0.5 mg/kg/day) in combination with tetracyclines (minocycline or doxycycline, 100 mg/day). Patients with generalized disease received first-line treatment including oral corticosteroids (1 mg/kg/day) in combination with tetracyclines and second-line treatment including immunosuppressants (methotrexate or mycophenolate mofetil).

Clinical status was measured according to the definitions and outcome measures for BP recommended by an international panel of experts in 2011 (9). Complete remission (CR) was defined as the absence of new or established lesions or pruritic symptoms while the patient was off therapy or receiving minimal therapy for at least 2 months. Partial remission (PR) was defined as the presence of transient new lesions that healed within 1 week while the patient was off therapy or receiving minimal therapy for at least 2 months. No response (NR) was defined as the development of new non-transient lesions or continued extension of old lesions, failure of established lesions to heal, or continued pruritus despite treatment. Minimal therapy was considered a prednisone dose ≤10 mg/day and an adjuvant dose of less than half of the therapeutic dose (e.g. minocycline 50 mg or mycophenolate mofetil 1 g).

Biopsy specimen slides stained with haematoxylin and eosin were reviewed, and the number of eosinophils was evaluated by an expert dermatopathologist. Data regarding the evaluation of eosinophil numbers in biopsy samples of patients with BP are not available in the literature. Therefore, the current study applied a method that is used for other eosinophilic diseases, such as eosinophilic oesophagitis and eosinophilic colitis. With these diseases, as part of the diagnosis, the eosinophilic count is evaluated using high-power fields (HPFs; ×400) (19). In the current study, eosinophils were counted in 5 random HPFs of the papillary and superficial reticular dermis to overcome possible variations in eosinophil infiltration. The mean count was used for further eosinophil analyses of the biopsy sample. The eosinophil count was categorized as no eosinophilic infiltrate (mean eosinophil count <1 cell per HPF), poor eosinophilic infiltrate (mean eosinophil count ≤12 cells per HPF), and rich eosinophilic infiltrate (mean eosinophil count >12 cells per HPF). Patients were divided into 3 groups: (group 1) no tissue eosinophils, (group 2) poor tissue eosinophils count and (group 3) rich tissue eosinophils count.

Statistical analysis

Statistical analysis was conducted using R (version 3.6.3; R Foundation, Vienna, Austria; R Core Team. R: A language and environment for statistical computing, 2020. https://www.R-project.org/). The statistical significance of the differences in means of continuous variables was calculated using Student's *t*-test for comparisons of 2 groups; a 1-way analysis of variance was used for comparisons of 2 or more groups. Categorical variable associations were analysed using Pearson's χ^2 test. The Yates continuity correction was applied to contingency tables containing cells with 5 or fewer samples. Statistical significance was set at $p<0.05$. Logistic regression models were fitted using R "base" package.

The response variable was determined as follows: NR=0 and response=1. Because the predictor variable mean tissue eosinophil count (tEOSc) distribution was heavily right-skewed, tEOSc was transformed using log10. To avoid infinite values for the transformation of tEOSc of 0, 0.1 was added to all values. Odds ratios were calculated using the "oddsratio" package in R (Schratz P 2017. R package 'oddsratio': odds ratio calculation for GAM(M)s & GLM(M)s, version: 1.0.2.).

RESULTS

Patients' characteristics

A total of 278 patients were diagnosed with BP between January 2012 and February 2020 at Sheba Medical Center. Of these, 141 patients were excluded; 15 due to an

Table I. Demographic characteristics and clinical and laboratory results of all bullous pemphigoid patients and the 3 individual groups[a]

	All patients $n = 137$	Group 1 $n = 42$	Group 2 $n = 64$	Group 3 $n = 31$	p-value
Demographic characteristics					
Age, years, mean (SD)	75.23 (12.6)	77.05 (10)	73.5 (12.8)	76.35 (14.8)	0.31
Females, *n* (%)	77 (56.2)	24 (57.14)	34 (53.13)	19 (61.29)	0.75
Comorbidities, *n* (%)					
Cardiac illness	77 (56.2)	21 (50)	38 (59.38)	18 (58.06)	0.62
Neoplasm	24 (17.52)	8 (19.05)	11 (17.19)	5 (16.13)	0.94
Neurological illness	39 (28.47)	7 (16.67)	17 (26.56)	15 (48.39)	0.011
Autoimmune disease	22 (16.06)	8 (19.05)	13 (20.31)	1 (3.22)	0.085
Diabetes mellitus	68 (49.64)	24 (57.14)	31 (48.44)	13 (41.94)	0.42
Dipeptidyl peptidase-4 inhibitor-associated disease	23 (16.79)	5 (11.9)	13 (20.31)	5 (16.13)	0.52
Clinical picture at presentation					
Blisters, *n* (%)	106 (77.37)	33 (78.57)	49 (76.56)	24 (77.42)	0.97
Oral involvement, *n* (%)	30 (21.9)	12 (28.57)	14 (21.88)	4 (12.9)	0.28
Body surface area, %, mean (SD)	58.4 (25.12)	58.36 (23.63)	59.73 (26.25)	55.63 (25.32)	0.81
Peripheral eosinophilia, *n* (%)	51 (39.84) *$n = 128$	6 (15.38) *$n = 39$	25 (41.67) *$n = 60$	20 (69) *$n = 29$	< 0.001
Treatment regimen, *n* (%)					
Topical corticosteroids	21 (15.32)	6 (14.28)	11 (17.18)	4 (12.9)	0.74
Tetracyclines	16 (11.67)	4 (9.52)	10 (15.62)	2 (6.45)	
Oral corticosteroid	23 (16.78)	7 (16.66)	10 (15.62)	6 (19.35)	
Oral corticosteroids with tetracyclines	71 (51.82)	22 (52.38)	30 (46.87)	19 (61.29)	
Immunosuppressants	6 (4.37)	3 (7.14)	3 (4.68)	0 (0)	

[a]Group 1: no eosinophilic infiltrates (mean eosinophil count < 1 cell per high-power field (HPF)). Group 2: poor eosinophilic infiltrates (mean eosinophil count ≤ 12 cells/HPF). Group 3: rich eosinophilic infiltrates (mean eosinophil count > 12 cells per HPF). SD: standard deviation. *This variable had a different number of patients who had their blood tested.

inconclusive diagnosis, 60 because they had no follow-up data, and 66 because their biopsy samples could not be re-evaluated. A final total of 137 patients were included in the study; 77 (56.2%) women and 60 (43.8%) men. Mean age at the time of diagnosis was 75.23 ± 12.5 years (range 33–99 years). Comorbidities of neurological illness, including Parkinson's disease, dementia, stroke, epilepsy, and multiple sclerosis, were found in 39 patients (28.47%). DPP-4i-associated BP was observed in 24 patients (17.5%). The clinical presentation and laboratory results of the study population at presentation showed that 106 patients (77.37%) had bullae, 107 patients (78.1%) had skin involvement only, and 30 patients (21.9%) had both skin and mucosal involvement. The mean BSA was 58.4 ± 25.12%. Peripheral eosinophilia was present in 51 patients (39.84%) (**Table I**). Patients were divided into 3 groups: group 1: no tissue eosinophils (mean eosinophil count < 1 cell per HPF); group 2: poor tissue eosinophils count (mean eosinophil count ≤ 12 cells per HPF); group 3: rich tissue eosinophils count (mean eosinophil count > 12 cells per HPF). Forty-two patients (30.6%) comprised group 1, 64 patients (46.7%) comprised group 2,

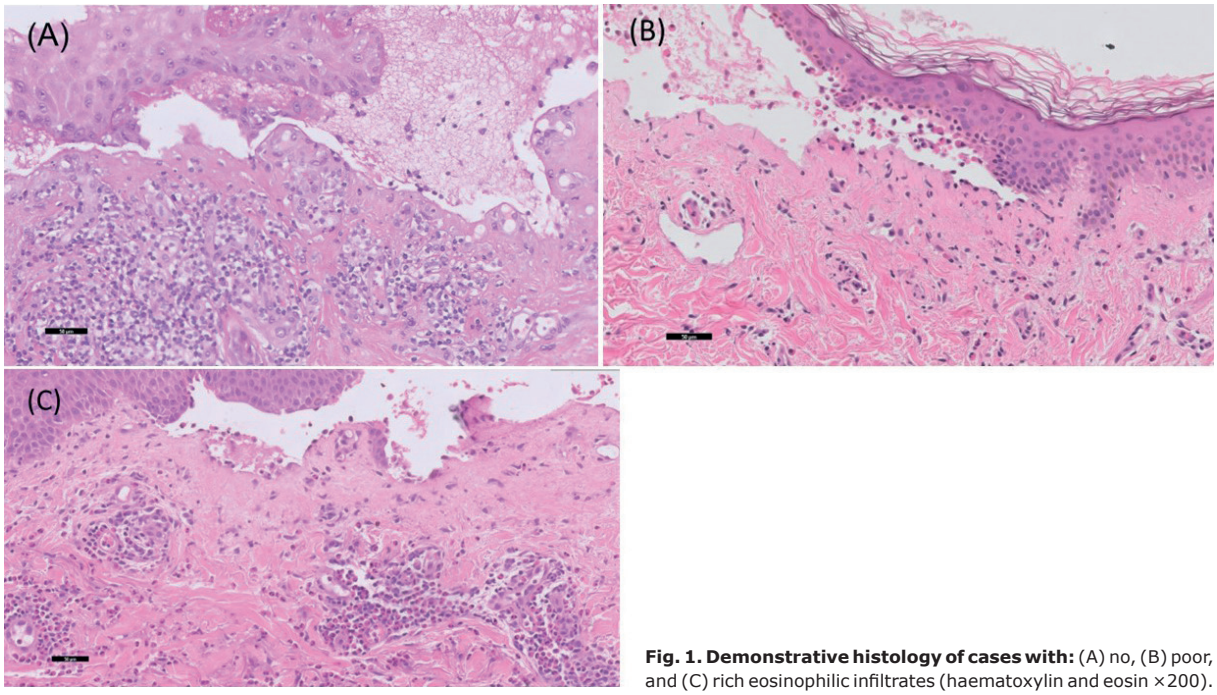

Fig. 1. Demonstrative histology of cases with: (A) no, (B) poor, and (C) rich eosinophilic infiltrates (haematoxylin and eosin ×200).

Table II. Response to treatment and follow-up of all patients with bullous pemphigoid (BP) and the 3 individual groups

	NR *n* (%)	PR *n* (%)	CR *n* (%)	*p*-value
Follow-up at 1 month				
All BP patients (N=137)	45 (32.85)	80 (58.39)	12 (8.76)	
Group 1 (N=42)	3 (7.14)	34 (80.95)	5 (11.91)	0.0008
Group 2 (N=64)	30 (46.88)	29 (45.31)	5 (7.81)	
Group 3 (N=31)	12 (38.71)	17 (54.84)	2 (6.45)	
Follow-up at 3 months				
All BP patients (N=119)	37 (31.09)	54 (45.38)	28 (23.53)	
Group 1 (N=35)	6 (17.14)	19 (54.29)	10 (28.57)	0.25
Group 2 (N=58)	21 (36.21)	23 (39.66)	14 (24.13)	
Group 3 (N=26)	10 (38.46)	12 (46.15)	4 (15.39)	
Follow-up at 6 months				
All BP patients (N=100)	22 (22)	43 (43)	35 (35)	
Group 1 (N=29)	1 (3.45)	15 (51.72)	13 (44.83)	0.009
Group 2 (N=52)	15 (28.85)	17 (32.69)	20 (38.46)	
Group 3 (N=19)	6 (31.58)	11 (57.89)	2 (10.53)	

Group 1: no eosinophilic infiltrates (mean eosinophil count < 1 cell per high-power field (HPF)). Group 2: poor eosinophilic infiltrates (mean eosinophil count ≤ 12 cells/HPF). Group 3: rich eosinophilic infiltrates (mean eosinophils count > 12 cells per HPF).
NR: no response; PR: partial response; CR. complete remission.

and 31 patients (22.6%) comprised group 3 (Table I; for examples see **Fig. 1**).

There were no significant differences in age or sex among the 3 groups ($p=0.31$ and $p=0.75$, respectively). Regarding medical history, the prevalence of neurological comorbidity was lowest in group 1 (16.7%), followed by group 2 (26.5%); it was highest in group 3 (48.4%) ($p=0.011$). No differences were found in other comorbidities, including DPP-4i-associated disease ($p=0.52$) (Table I). No differences in the percentage of patients with a blistering clinical picture ($p=0.97$), oral involvement ($p=0.28$), and BSA extent at disease onset ($p=0.81$) were observed among the 3 groups. There was a direct correlation between blood and tissue eosinophil counts. The proportion of patients with peripheral eosinophilia was highest in group 3 (69%), followed by group 2 (41.67%), and it was lowest in group 1 (15.38%) ($p<0.001$) (Table I).

Response to treatment at follow-up

At the 1-month follow-up, 45 (32.85%) patients had NR to treatment, 80 (58.39%) had PR to treatment, and 12 (8.76%) had CR to treatment. There was a significant association among the 3 groups and treatment response ($p=0.0008$) (**Table II**; **Fig. 2**); tissue eosinophil counts and treatment outcomes were negatively correlated. There were significantly more patients who achieved a response (PR or CR) in group 1 lacking tissue eosinophilic infiltrates than in groups 2 and 3 (92.86% vs 55.79%), and significantly fewer patients with NR were in group 1 than in groups 2 and 3 (7.14% vs 44.21%; $p<0.0001$) (**Table III**; Fig. 2). The comparison of groups 2 and 3 revealed no significant differences in the response to treatment ($p=0.68$).

At the 3-month follow-up, 18 patients (1.3%) were lost to follow-up; therefore, 119 patients were evaluated. Results showed that 37 patients (31.09%) had NR to treatment, 54 (45.38%) had PR to treatment, and 28 (23.53%) had CR to treatment. There was no significant association among the 3 groups and the treatment response at this follow-up point ($p=0.25$) (Table II; Fig. 2). However, there were considerably more patients who achieved a response (PR or CR) in group 1 than in groups 2 and 3 (82.86% vs 63.1%), and fewer patients with NR to treatment were in group 1 than in groups 2 and 3 (17.14% vs 36.9%), which tended toward significance ($p=0.056$). (Table III; Fig. 2). The comparison of groups 2 and 3 revealed no significant differences in the response to treatment ($p=0.65$).

At the 6-month follow-up, 19 patients (15%) were lost to follow-up; therefore, 100 patients were evaluated. Re-

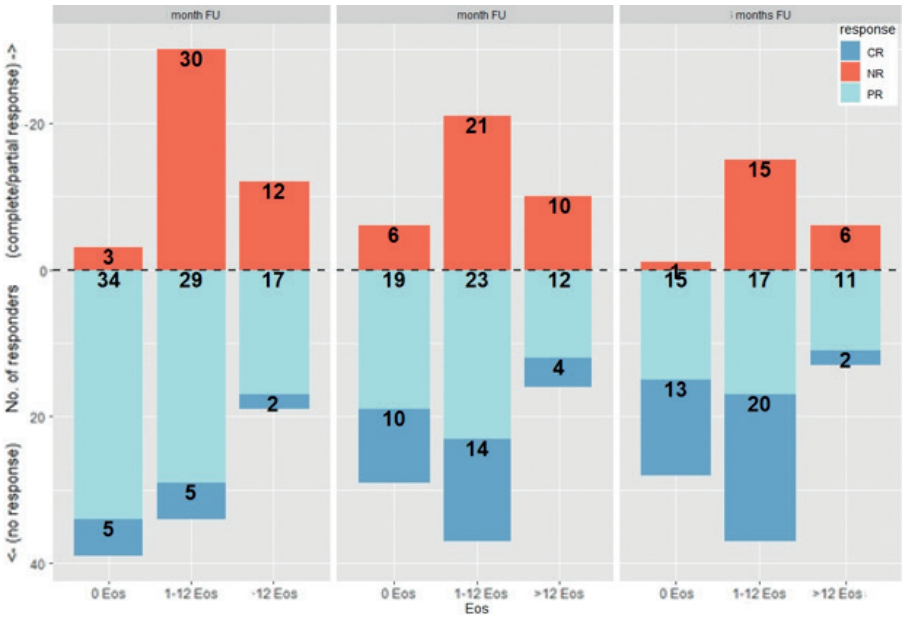

Fig. 2. Differential response rates of the 3 eosinophilic groups at 3 different follow-up (FU) points. (A) At 1-month FU, the χ^2 test showed that there was a significant association among the 3 groups and the treatment response ($p=0.0008$). In addition, significantly more patients achieved a response (partial remission (PR) or complete remission (CR)) in group 1 than in groups 2 and 3 (*bottom green bracket*, $p<0.0001$). (B) At 3-month FU, a χ^2 test showed that there was no significant association between the histological group and the treatment response ($p=0.25$). However, there were significantly more patients who achieved a response in group 1 than in groups 2 and 3 (*top green bracket*, $p=0.056$). (C) At 6-month FU, a χ^2 test showed a significant association among the 3 groups and the clinical response ($p=0.009$). Significantly more patients who achieved CR in group 2 than in group 3 (*bottom green bracket*, $p=0.02$).

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Table III. Response to treatment and follow-up of group 1 compared with groups 2 and 3

	No response *n* (%)	Partial response+ complete remission *n* (%)	*p*-value
Follow-up at 1 month			
Group 1 (N=42)	3 (7.14)	39 (92.86)	<0.0001
Groups 2 and 3 (N=95)	42 (44.21)	53 (55.79)	
Follow-up at 3 months			
Group 1 (N=35)	6 (17.14)	29 (82.86)	0.056
Groups 2 and 3 (N=84)	31 (36.9)	53 (63.1)	
Follow-up at 6 months			
Group 1 (N=29)	1 (3.45)	28 (96.55)	0.009
Groups 2 and 3 (N=71)	21 (29.58)	50 (70.42)	

Group 1: no eosinophilic infiltrates (mean eosinophil count <1 cell per high-power field (HPF)). Groups 2 and 3: poor and rich eosinophilic infiltrates (mean eosinophil count >1 cell per HPF).

sults showed that 22 patients (22%) had NR to treatment, 43 (43%) had PR to treatment, and 35 (35%) had CR to treatment. There was a significant association among the 3 groups and the treatment response ($p=0.0008$) (Table II; Fig. 2). The tissue eosinophil counts and treatment outcomes were negatively correlated. There were significantly more patients who achieved a response (PR or CR) in group 1 than in groups 2 and 3 (96.55% vs 70.42%), and there were significantly fewer patients with NR to treatment in group 1 than in groups 2 and 3 (3.45% vs 29.58%; $p=0.009$). (Table III; Fig. 2). The comparison of groups 2 and 3 revealed that significantly more patients achieved CR to treatment in group 2 (10.53% vs 40.74%; $p=0.02$) (Fig. 2).

Similar results were found in a sub-analysis from which patients with DPP-4i associated disease were excluded, with a significant association among the 3 groups and treatment response at the 1-month ($p=0.001$) and 6-month follow-ups ($p=0.05$). Tissue eosinophil counts and treatment outcomes were negatively correlated. Similarly to the previous results, at the 3-month follow-up, there were no significant associations between the 3 groups and the treatment responses. However, there were considerably more patients who achieved a response (PR or CR) in group 1 than in groups 2 and 3 (80.65% vs 64.6%) ($p=0.1$).

To further assess the potential use of tEOSc as a biomarker for the treatment response with BP, a single-variable logistic regression model was used. Because differences in the response to treatment were most significant at the 6-month follow-up, this time interval was used to fit the model that predicted the treatment response probability as a function of tEOSc. The log10 tEOSc coefficient was –0.784 and showed statistical significance ($p=0.0306$). The odds ratio for tEOSc was 0.457 (95% confidence interval (95% CI) 0.212–0.892), indicating that an increase of 1 log10 (i.e. 10-fold) in tEOSc was associated with a reduced probability of treatment response by a factor of 0.457. Then, practical tEOSc cut-offs for response prediction were determined in the following manner: the response proportions below and above each possible unique cut-off point were calculated. Thereafter, the difference in the response proportion for the cut-offs was calculated. This calculation indicated that, with tEOSc of 19, the treatment response rate decreased from 81.1% (73/90 patients) to 50% (5/10 patients). Then, the same procedure was performed for the remaining group of patients, which indicated that with tEOSc of 0.8, the treatment response rate decreased from 96.55% (28/29 patients) to 73.8% (45/61 patients) (**Fig. 3**).

To eliminate possible confounders, a multivariate logistic regression analysis was conducted. The model predicted CR at 6 months and included the patient's age at diagnosis, sex, comorbidity of neurological illness, DPP4-i associated disease, peripheral eosinophilia, tissue eosinophil groups and treatment regimen as predictors. Only the tissue eosinophil group was significantly associated with CR (OR –0.29, 95% CI –0.55 to –0.03, $p=0.03$) (**Fig. 4**).

DISCUSSION

This study included 137 patients with BP, with a mean age of 75.2 years, which was compatible with the literature (1). A previous study showed that the prevalence of neurological comorbidity for patients with BP ranged from 28% to 56% (2). Similarly, in our study, 28.47% of patients had neurological illness. Gambichler et al. found that the presence of neurological disease was significantly correlated with blood eosinophil counts (20). Neurological diseases were significantly more common in patients with BP with high levels of eosinophils in the tissue. This finding is consistent with the results of a recent study by Karaali et al. (21), which demonstrated a correlation

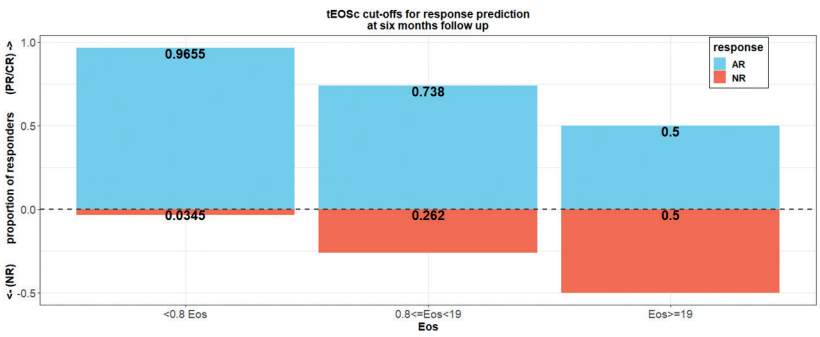

Fig. 3. Tissue eosinophil count (tEOSc) cut-offs for the response prediction at 6-month follow-up. The tEOSc was defined as the mean number of eosinophils detected in 5 different, randomly chosen, microscopic high-power fields (×40) of tissue samples. Response proportions were calculated below and above each unique tEOSc value, and the most practical cut-off points were chosen as cut-offs (< 0.8 eosinophils, 0.8–19 eosinophils, and > 19 eosinophils, respectively). Clinical response groups were divided into no response (NR; *red*) and any response (AR; *blue*). The < 0.8 eosinophils group had a response rate of 96.55%. The 0.8–19 eosinophils group had a response rate of 73.8%. The > 19 eosinophils group had a response rate of 50%.

Variable		N	Estimate	p
DPP-4i associated_disease	0	76	Reference	
	1	19	-0.18 (-0.44, 0.08)	0.17
Sex	male	43	Reference	
	female	52	0.04 (-0.17, 0.24)	0.73
Neurological_illness	0	73	Reference	
	1	22	-0.05 (-0.30, 0.20)	0.71
Peripheral_eosinophilia (cells/dL)	0	61	Reference	
	1	34	0.08 (-0.12, 0.29)	0.43
Treatment_regimen	Oral_corticosteroids	16	Reference	
	Oral_corticosteroids_with_Tetracycline	52	-0.08 (-0.36, 0.20)	0.59
	Tetracycline	13	-0.01 (-0.37, 0.35)	0.97
	Immunosuppresant	6	-0.33 (-0.79, 0.13)	0.17
	Topical_corticosteroids	8	-0.30 (-0.72, 0.12)	0.17
Rich_eosinophilic_infiltrate	0	78	Reference	
	1	17	-0.28 (-0.55, -0.02)	0.04
Age_at_diagnosis (>70)	0	31	Reference	
	1	64	0.11 (-0.11, 0.33)	0.32

Fig. 4. Multivariate logistic regression analysis for predicting complete remission (CR) at 6 months. DPP-4i, dipeptidyl peptidase-4 inhibitor. 0: no. 1: yes.

between patients with BP with neurological disease and high tissue eosinophil counts. The study suggested that eosinophils may have a common role in the pathogenesis of BP and neurological diseases; however, further studies are needed to prove this theory (21). No differences were found in the prevalence of other comorbidities, including cardiac disease, diabetes, malignancy, and autoimmunity. Karaali et al. (21) also showed that there was no association between malignancy and tissue eosinophil levels; however, they did not assess other comorbidities. There are no other studies in the literature that have evaluated the relationship between comorbidities associated with BP and skin eosinophil counts. Of note, although, however, there is some evidence to suggest that BP related to DPP-4i displays unique clinical and immunological features, such as non-inflammatory phenotypes and different immunodominant BP180 epitopes targeted by the antibodies (22). The current study results suggest that, despite the clinical and immunological differences that may exist, no pathological difference exists.

At presentation, the mean BSA was 57.8%, and 77.9% of patients presented with blistering. This correlated with the results of a study by Torre et al., which showed that there were no bullae at the time of diagnosis in up to 20% of patients with BP (23). There was no significant difference in the mean BSA involved with the disease or in the percentage of patients with a blistering clinical picture among the 3 groups with different amounts of tissue eosinophils infiltrate. This was in contrast to the results of a study by Farnaghi et al. (24), which demonstrated a correlation between dermal eosinophilia and BPDAI. Karaali et al. (21) also showed that BPDAI scores were higher for patients with high tissue eosinophil levels. This could be explained by the differences in the ethnic and geographical characteristics of the participants, and by the different sample sizes in the current study.

In the current study, 19.7% of patients had oral involvement. This correlated with the results of the study by Amber et al. (2), which showed mucosal involvement in 10–20% of patients and with the study by Ständer et al. (25). There was no significant difference in the percentage of patients with oral involvement among the 3 groups. This finding is consistent with those of Farnaghi et al. (24), who did not find any significant correlation between the severity of mucosal involvement and tissue eosinophilia. Peripheral eosinophilia was noted in 42.1% of the patients, which was similar to the percentage reported by Amber et al. (2). Our knowledge of the relationship between tissue and blood eosinophil levels in patients with BP is limited. The current data indicated a significant correlation between the number of eosinophils in the skin and peripheral eosinophilia. This finding is inconsistent with those of the study by Karaali et al. (21), which did not find that correlation. However, we believe that our findings are more logical and reliable. The current study included a large sample size of 137 participants. Both blood work and biopsies were performed during presentation of the disease. The biopsy samples were re-evaluated by an expert dermatopathologist. The clinical data were collected by another expert dermatologist who was blinded to the results of the biopsy samples.

In terms of the treatment response, the current findings revealed consistently better response to treatment in group 1 (no tissue eosinophils infiltrate) at 3 different follow-up points.

Regarding the comparison between groups 2 (poor tissue eosinophils count) and 3 (rich tissue eosinophils count): at the 1-month and 3-month follow-up evaluations, no difference was found in the response to therapy. However, at the 6-month follow-up evaluation, group 3 had significantly lower CR rates.

In addition, at the 6-month follow-up evaluation, an increase of 1 $\log_{10}$ (i.e. 10-fold) in the mean eosinophil count in the tissue was associated with a reduced probability of a response to treatment.

These findings indicate that, at the 1-month and 3-month follow-up points, the response to treatment

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was based on the presence of eosinophils in the skin, regardless of the count. However, at the 6-month follow-up point, the following was observed: the higher the eosinophil count, the less the response to treatment. This suggests that changes in the response to treatment among eosinophilic groups are more clearly expressed at a later stage of the disease.

To date, only 2 studies have examined the association between BP severity and histopathological parameters. Farnaghi et al. (24) performed a prospective study that included 27 patients with BP and found correlations among anti-BP-180, anti-BP-230, dermal eosinophilia, and tissue inflammation severity with BPDAI scores. A retrospective study by Karaali et al. (21) including 59 patients found that BPDAI scores were higher in those with high tissue eosinophil levels. However, there was no significant difference between groups in terms of disease activity control and time to achieving CR during therapy. CR on and off therapy was similar in both groups during the course of disease. This is in contrast to the results of the current study, which showed a significant difference in the response to treatment among the eosinophilic groups.

The strength of this study was the large number of patients with the full spectrum of skin eosinophil counts.

This study has a number of limitations. It was a retrospective study, and lacked information regarding the patients' anti-BP180 NC16A antibody titres, since indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) are not part of the routine BP workup in our centre. Another limitation is the follow-up duration (6 months), and that not all patients participated during the full follow-up period.

In conclusion, during this study, although skin eosinophil counts were not correlated with disease severity at presentation, there was a significant negative correlation with treatment response and the chance of achieving CR.

The authors have no conflicts of interest to declare.

REFERENCES

1. Baigrie D, Nookala V. Bullous pemphigoid. StatPearls. 2021 Dec 5 [cited 16 January 2022]. Available from: https://www-ncbi-nlm-nih-gov.beilinson-ez.medlcp.tau.ac.il/books/NBK535374.
2. Amber KT, Murrell DF, Schmidt E, Joly P, Borradori L. Autoimmune subepidermal bullous diseases of the skin and mucosae: clinical features, diagnosis, and management. Clin Rev Allergy Immunol 2018; 54: 26–51.
3. Bernard P, Antonicelli F. Bullous pemphigoid: a review of its diagnosis, associations and treatment. Am J Clin Dermatol 2017; 18: 513–528.
4. Simon D, Borradori L, Simon HU. Eosinophils as putative therapeutic targets in bullous pemphigoid. Exp Dermatol 2017; 26: 1187–1192.
5. Ständer S, Hammers CM, Vorobyev A, Schmidt E, Zillikens D, Ghorbanalipoor S, et al. The impact of lesional inflammatory cellular infiltrate on the phenotype of bullous pemphigoid. J Eur Acad Dermatol Venereol 2021; 35: 1702–1711.
6. Bernard P, Venot J, Constant F, Bonnetblane JM. Blood eosinophilia as a severity marker for bullous pemphigoid. J Am Acad Dermatol 1987; 16: 879–881.
7. Zuo YG, Liu B, Li L, Jin HZ, Sun QN. [Correlation between blood eosinophil level and steroid doses in patients with bullous pemphigoid]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2012; 34: 130–133.
8. Kridin K. Peripheral eosinophilia in bullous pemphigoid: prevalence and influence on the clinical manifestation. Br J Dermatol 2018; 179: 1141–1147.
9. Murrell DF, Daniel BS, Joly P, Borradori L, Amagai M, Hashimoto T, et al. Definitions and outcome measures for bullous pemphigoid: recommendations by an international panel of experts. J Am Acad Dermatol 2012; 66: 479–485.
10. Delli FS, Sotiriou E, Lazaridou E, Apalla Z, Lallas A, Vakirlis E, et al. Total IgE, eosinophils, and interleukins 16, 17A, and 23 correlations in severe bullous pemphigoid and treatment implications. Dermatol Ther 2020; 33: e13958.
11. Giusti D, Gatouillat G, Le Jan S, Plée J, Bernard P, Antonicelli F, et al. Eosinophil cationic protein (ECP), a predictive marker of bullous pemphigoid severity and outcome. Sci Rep 2017; 7: 4833.
12. de Graauw E, Sitaru C, Horn M, Borradori L, Yousefi S, Simon HU, et al. Evidence for a role of eosinophils in blister formation in bullous pemphigoid. Allergy 2017; 72: 1105–1113.
13. Amber KT, Valdebran M, Kridin K, Grando SA. The role of eosinophils in bullous pemphigoid: a developing model of eosinophil pathogenicity in mucocutaneous disease. Front Med 2018; 5: 201.
14. Simon D, Yousefi S, Cazzaniga S, Bürgler C, Radonjic S, Houriet C, et al. Mepolizumab failed to affect bullous pemphigoid: a randomized, placebo-controlled, double-blind phase 2 pilot study. Allergy 2020; 75: 669–672.
15. Feng S, Wu Q, Jin P, Lin L, Zhou W, Sang H, et al. Serum levels of autoantibodies to BP180 correlate with disease activity in patients with bullous pemphigoid. Int J Dermatol 2008; 47: 225–228.
16. Koga H, Teye K, Ishii N, Ohata C, Nakama T. High index values of enzyme-linked immunosorbent assay for bp180 at baseline predict relapse in patients with bullous pemphigoid. Front Med 2018; 5: 139.
17. Liu Y, Wang Y, Chen X, Jin H, Li L. Factors associated with the activity and severity of bullous pemphigoid: a review. Ann Med 2020; 52: 55–62.
18. Feliciani C, Joly P, Jonkman MF, Zambruno G, Zillikens D, Ioannides D, et al. Management of bullous pemphigoid: the European Dermatology Forum consensus in collaboration with the European Academy of Dermatology and Venereology. Br J Dermatol 2015; 172: 867–877.
19. Collins MH, Capocelli K, Yang GY. Eosinophilic gastrointestinal disorders pathology. Front Med 2018; 4: 261.
20. Gambichler T, Segert H, Höxtermann S, Schmitz L, Altmeyer P, Teegen B. Neurological disorders in patients with bullous pemphigoid: clinical and experimental investigations. J Eur Acad Dermatol Venereol 2015; 29: 1758–1762.
21. Gore Karaali M, Koku Aksu AE, Cin M, Leblebici C, Kara Polat A, Gurel MS. Tissue eosinophil levels as a marker of disease severity in bullous pemphigoid. Australas J Dermatol 2021; 62: e236–e241.
22. Izumi K, Nishie W, Mai Y, Wada M, Natsuga K, Ujiie H, et al. Autoantibody profile differentiates between inflammatory and noninflammatory bullous pemphigoid. J Invest Dermatol 2016; 136: 2201–2210.
23. Della Torre R, Combescure C, Cortés B, Marazza G, Beltraminelli H, Naldi L, et al. Clinical presentation and diagnostic delay in bullous pemphigoid: a prospective nationwide cohort. Br J Dermatol 2012; 167: 1111–1117.
24. Farnaghi F, Amir-Hooshang E, Kamyab-Hesary K, Abbasian S, Seirafi H, Nasimi M. Correlation of dermal and blood eosinophilia with bullous pemphigoid disease severity. Int J Womens Dermatol 2020; 6: 171–175.
25. Ständer S, Schmidt E, Zillikens D, Ludwig RJ, Kridin K. Immunological features and factors associated with mucocutaneous bullous pemphigoid – a retrospective cohort study. J Dtsch Dermatol Ges 2021; 19: 1289–1295.