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ORIGINAL ARTICLE

Association of Peripheral Blood and Cutaneous Eosinophils With Bullous Pemphigoid Disease Severity and Treatment Outcomes



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KEYWORDS

Bullous dermatosis;
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Eosinophilia;
Eosinophils

Abstract

Background and aims: A dermal inflammatory infiltrate rich in eosinophils is a prominent histological feature of bullous pemphigoid (BP) and peripheral blood eosinophilia has been documented in 50–60% of BP patients. Nevertheless, the impact of circulating and dermal infiltrate eosinophil levels on BP remains poorly understood. The main objective of this work was to investigate the association of peripheral blood and dermal infiltrate eosinophil levels with clinical and immunological characteristics of the disease.

Material and methods: Retrospective cohort study including all patients diagnosed with BP between 2011 and 2020.

Results: The study cohort included 233 patients with BP. The mean baseline peripheral blood eosinophil count was $956.3 \pm 408.6 \times 10^6/L$ and the mean number of tissue eosinophils at the dermal hot spot area was 30.5 ± 19.0 . Patients with disseminated presentation (i.e. BSA > 50%) had significantly higher peripheral blood eosinophil counts ($P=0.028$). Mucosal involvement was significantly associated with lower dermal eosinophil count ($P=0.001$). Requiring inpatient care and relapsing were significantly associated with high peripheral blood eosinophil count ($P=0.025$; $P=0.020$, respectively). Among the 68 patients who experienced a relapse, 31 had peripheral blood eosinophilia (i.e. $>500 \times 10^6/L$) at relapse (44.2%). Peripheral blood eosinophil counts at baseline and at relapse were significantly correlated ($r=0.82$, $P<0.001$).

Conclusions: Peripheral blood and cutaneous eosinophils levels may be useful biomarkers for disease activity and treatment outcomes in BP. Monitoring peripheral blood eosinophil counts may allow early detection of relapse.

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PALABRAS CLAVE

Dermatosis
ampollosa;
Penfigoide ampolloso;
Eosinofilia;
Eosinófilos

Asociación entre eosinófilos de sangre periférica y cutáneos con la gravedad del penfigoide ampolloso y los resultados terapéuticos**Resumen**

Antecedentes y objetivos: El infiltrado inflamatorio dérmico rico en eosinófilos es una característica histológica destacada de penfigoide ampolloso (PA) y eosinofilia en sangre periférica, que se ha documentado en el 50-60% de los pacientes con esta enfermedad. Sin embargo, el impacto de los niveles de eosinófilos circulantes y en infiltrados dérmicos en el PA sigue sin comprenderse. El objetivo principal de este estudio fue investigar la asociación entre los niveles de eosinófilos en sangre periférica y en infiltrados dérmicos y las características clínicas e inmunológicas de esta enfermedad.

Material y métodos: Estudio de cohorte retrospectivo que incluyó a todos los pacientes con PA entre 2011 y 2020.

Resultados: El estudio de cohorte incluyó 233 pacientes con PA. El recuento de eosinófilos basal medio en sangre periférica fue de $956,3 \pm 408,6 \times 10^6/L$ y el número medio de eosinófilos tisulares en la zona dérmica clave fue de $30,5 \pm 19$. Los pacientes con presentación diseminada (es decir, BSA >50%) tuvieron conteos de eosinófilos en sangre periférica significativamente superiores ($p = 0,028$). El compromiso mucoso estuvo significativamente asociado a un conteo de eosinófilos cutáneo inferior ($p = 0,001$). La necesidad de cuidados hospitalarios y las recaídas estuvieron significativamente asociadas a conteos de eosinófilos en sangre periférica más elevados ($p = 0,025$; $p = 0,020$, respectivamente). Entre los 68 pacientes que experimentaron recidiva, 31 tuvieron eosinofilia en sangre periférica (es decir, $> 500 \times 10^6/L$) en recaída (44,2%). Los conteos de eosinófilos en sangre periférica basales y en recaída se correlacionaron significativamente ($r = 0,82$, $p < 0,001$).

Conclusiones: Los niveles de eosinófilos en sangre periférica y cutáneos pueden constituir biomarcadores útiles para la actividad de la enfermedad y los resultados terapéuticos en el PA. Supervisar los conteos de eosinófilos en sangre periférica puede ayudar a detectar la recidiva tempranamente.

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Introduction

Bullous pemphigoid (BP) is the most common autoimmune subepidermal blistering disease.¹ BP affects mostly elderly patients and is associated with significant rates of morbidity and mortality.² In the past two decades a trend of growing incidence between 1.9 and 4.3-fold has been reported in data from different European countries.^{3,4} The increased incidence of BP overtime has been associated with an ageing population, with the increased prevalence of neurologic diseases and with the increasing use of drugs associated with BP development, in particular dipeptidyl peptidase-IV (DPP-4) inhibitors.⁵

Several observations support a pathogenic role of eosinophils in BP. Dermal inflammatory infiltrate rich in eosinophils is a prominent histological feature and peripheral blood eosinophilia has been documented in 50–60% of BP patients.^{6,7} Moreover, peripheral blood and cutaneous eosinophil levels have been correlated with disease severity.^{7,8} Eosinophils seem to be key effector cells in BP initiation and progression. In fact, eosinophil skin infiltration is an early event and precedes blister formation.⁹ Eosinophils degranulation and release of extracellular eosinophil traps and granule proteins promote lesion formation.^{10,11}

Although increasing evidence of contribution of eosinophils for BP pathogenesis accumulate, the influence of circulating and dermal infiltrate eosinophil levels

on the phenotype of BP remains poorly understood. The main objective of this study was to evaluate the association between peripheral blood and cutaneous eosinophil levels and the clinical and immunological characteristics of the disease.

Materials and methods

A retrospective cohort study was conducted on all patients diagnosed with BP between January 1st, 2011 and December 31st, 2020, at a tertiary referral centre. The study was approved by the Local Ethics Committee.

The diagnosis of BP was established according to the following criteria: (1) suggestive clinical and histopathological picture; (2) linear deposits of IgG and/or C3 along the dermal–epidermal junction by direct immunofluorescence (DIF) microscopy of a perilesional skin biopsy; and (3) the presence of circulating IgG autoantibodies against BP180 and/or BP230, as identified by BP180 NC16A/BP230 enzyme-linked immunosorbent assay (ELISA).

We excluded all patients with an alternative cause for peripheral blood and tissue eosinophilia, including atopic dermatitis, asthma, allergic rhinitis, parasitic infection and lymphoma. All patients treated with drugs that affect eosinophil counts in the moment of BP diagnosis, including systemic steroids and immunosuppressants, were also excluded.

Peripheral blood eosinophil counts prior to the administration of any treatment were reviewed. Peripheral blood eosinophil counts were classified as normal if below $500 \times 10^6/L$, high if between 500 and $1500 \times 10^6/L$ and very high if above $1500 \times 10^6/L$. The levels of peripheral blood anti-BP180 NC16A and anti-BP230 IgG autoantibodies were measured by ELISA (Euroimmun, Lübeck, Germany). Seropositivity was determined based on the cut-off values proposed by the manufacturer (i.e., 20 U/ml).

Haematoxylin and eosin (H&E)-stained lesional skin specimens were reviewed in terms of eosinophil levels by three Dermatopathologists (MAG, PEL, LSA). In each sample, representative hot spots with the highest density of eosinophils were identified in the dermal inflammatory infiltrate. In each hot spot the number of eosinophils was counted using a single $\times 400$ high power field (HPF) objective (one large magnification area hot spot).

Data was analysed using IBM SPSS Statistics® (Statistical Package for the Social Sciences, version 24, SPSS Inc., Chicago, IL, USA). Categorical variables were presented as frequencies and percentages and were compared with use of Chi-square test. Data found to be non-normally distributed were analysed using the Mann–Whitney *U* test for independent subgroups and the Wilcoxon test for dependent subgroups. Continuous variables were presented as means and standard deviations. These variables were compared with the use of Student's *t*-test. All reported *P* values are two-tailed, with a *P* value of 0.05 indicating statistical significance.

Results

Descriptive characteristics and comorbidities of the study population

The study cohort included 233 patients with BP, of whom 123 (52.8%) were males. The mean age (SD) at diagnosis was 79.3 (9.3) years and the median age was 81.0 (range 51.0–101.0).

Overall, 113 patients (48.5%) had type-2 diabetes mellitus at the onset of BP. Seventy-two patients (30.9%) developed BP while being treated with DPP-4 inhibitors. The most commonly prescribed DPP-4 inhibitor was vildagliptin ($n=36$; 50.0%), followed by linagliptin ($n=24$; 33.3%) and sitagliptin ($n=12$; 16.7%). The median latency time between the DPP-4 inhibitor introduction and the onset of BP was 18.0 months (range 4.0–96.0 months).

Ninety-three patients (39.9%) had concomitant neurologic disease. The most common associated neurologic disease was dementia ($n=48$; 20.6%), followed by cerebrovascular disease ($n=31$; 13.3%), Parkinson disease ($n=16$; 6.9%) and epilepsy ($n=9$; 3.9%).

Descriptive characteristics of the patients are detailed in Table 1.

Association between peripheral blood and cutaneous eosinophil levels and features of BP

The mean peripheral blood eosinophil count was $956.3 \pm 408.6 \times 10^6/L$. At the dermal hot spot area, the mean number of eosinophils was 30.5 ± 19.0 . Dermal

Table 1 Demographic, clinical, and immunological characteristics of BP patients.

Descriptive characteristics	
<i>Age at diagnosis, years</i>	
Mean (SD)	79.3 (9.3)
<i>Sex, n (%)</i>	
Male	123 (52.8%)
<i>Comorbidities, n (%)</i>	
Type 2 diabetes mellitus	113 (48.5%)
Neurologic disease	93 (39.9%)
Malignancy	33 (14.2%)
<i>DPP-4 inhibitor-associated BP, n (%)</i>	
Vildagliptin	37 (15.9%)
Linagliptin	23 (9.9%)
Sitagliptin	12 (5.1%)
<i>Anti-PD-1-associated BP, n (%)</i>	
	1 (0.4%)
<i>Distribution of bullous lesions; n (%)</i>	
Upper limbs	205 (88.0%)
Lower limbs	202 (86.7%)
Trunk	199 (85.4%)
Hands and feet	169 (72.5%)
Head and neck	57 (24.5%)
Mucosal involvement	39 (16.7%)
Disseminated presentation (i.e. BSA > 50%)	113 (48.5%)
<i>Peripheral blood eosinophil count, baseline ($\times 10^6/L$)</i>	
Mean (SD)	956.3 (408.6)
<i>Peripheral blood eosinophil levels, baseline; n (%)</i>	
Normal ($<500 \times 10^6/L$)	96 (41.2%)
High ($500\text{--}1500 \times 10^6/L$)	114 (48.9%)
Very high ($>1500 \times 10^6/L$)	23 (9.9%)
<i>Dermal infiltrate eosinophil count (n. cels/hot spot area)</i>	
Mean (SD)	30.5 (19.0)
<i>Dermal infiltrate eosinophil levels; n (%)</i>	
No (0)	8 (3.4%)
Low (<25)	75 (32.2%)
Intermediate (25–50)	67 (28.8%)
High (>50)	83 (35.6%)
<i>Anti-BP180 NC16A IgG ELISA</i>	
ELISA value, mean (SD); (U/ml)	128.4 (85.2)
<i>Anti-BP230 IgG ELISA</i>	
ELISA value, mean (SD); (U/ml)	32.0 (27.4)
<i>Linear deposits of immunoreactants by direct immunofluorescence, n (%)</i>	
C3	226 (97.0%)
IgG	208 (89.3%)
IgA	17 (7.3%)
IgM	5 (2.1%)
Isolated C3	18 (7.7%)
<i>Treatment; n (%)</i>	
Oral prednisone (>0.5 mg/Kg/day)	219 (94.0%)
Tetracycline	109 (46.8%)
Adjuvant immunosuppressant	74 (31.8%)
Isolated topical treatment	5 (2.1%)

Table 1 (Continued)

Number of treatment lines	
Mean (SD)	1.8 (1.0)
Inpatient care; n (%)	89 (39.2%)
Relapsing; n (%)	68 (29.2%)
Peripheral blood eosinophil count, relapse (x10⁶/L)	
Mean (SD)	949.4 (412.0)
Peripheral blood eosinophil levels, relapse; n (%)	
Normal (<500 × 10 ⁶ /L)	37 (54.4%)
High (500–1500 × 10 ⁶ /L)	19 (27.9%)
Very high (>1500 × 10 ⁶ /L)	12 (17.6%)

BP: bullous pemphigoid, DPP4: dipeptidyl peptidase-4, ELISA: enzyme-linked immunosorbent assay, *n*: number, SD: standard deviation.

infiltrate eosinophil number were directly correlated with peripheral blood eosinophil counts ($r=0.234$, $P=0.004$). The relationship between peripheral blood and dermal infiltrate eosinophil counts and demographic, clinical, and immunological characteristics of BP patients is detailed in Table 2.

Dermal infiltrate eosinophil counts were significantly higher in patients with BP accompanying neurological disease ($P=0.032$). In contrast, patients treated with DPP-4 inhibitors had significantly lower counts of eosinophils both at the peripheral blood and at the dermal hot spot area ($P=0.008$; $P=0.013$, respectively).

With respect to the anatomical distribution of lesions, presenting a disseminated form of BP (i.e. BSA > 50%) was significantly more frequent in the group with high peripheral blood eosinophil counts ($P=0.028$). Moreover, hands and feet involvement were also more frequent in this group ($P=0.029$). Mucosal involvement was significantly associated with lower eosinophil count in the dermal infiltrate hot spot area ($P=0.001$).

The levels of anti-BP180 NC16A IgG antibodies were directly correlated both with peripheral blood and with dermal infiltrate eosinophil counts ($r=0.356$, $P=0.001$; $r=0.197$, $P=0.43$, respectively). The mean level of anti-BP180 NC16A IgG antibodies was significantly higher in the group with high peripheral blood eosinophil counts ($P<0.001$). No correlation was found with anti-BP230 IgG antibodies.

Requiring inpatient care was significantly associated with higher baseline peripheral blood eosinophil count ($P=0.025$). Moreover, relapsing was also more frequent among patients with higher peripheral blood eosinophil counts at presentation ($P=0.020$). Most relapses occurred within the first six months of the study ($n=56/68$; 82.4%). Among the 68 patients who experienced a relapse of BP, 19 had high and 12 very high peripheral blood eosinophil counts at relapse (27.9% and 16.6%, respectively). In these patients, peripheral blood eosinophil counts at baseline and at relapse were significantly correlated ($r=0.82$, $P<0.001$). Most patients who relapsed and had high baseline peripheral blood eosinophil count also presented peripheral blood eosinophilia at relapse ($n=22/31$; 71.0%). In contrast, only two patients with non-elevated baseline peripheral blood

eosinophil count subsequently presented peripheral blood eosinophilia at relapse ($n=2/37$; 5.4%).

Discussion

The current study revealed that peripheral blood and cutaneous eosinophil levels are associated with BP severity and with treatment outcomes. Peripheral blood eosinophilia was associated with extensive disease and with hand and feet involvement. Moreover, it predicted a higher risk of requiring inpatient care and relapsing. Peripheral blood and tissue eosinophil levels were associated with high titles of BP180 NC16A IgG antibodies. Eosinophil-poor inflammatory infiltrates predicted an increased prevalence of mucosal involvement.

Previous studies had provided inconclusive conclusions on the influence of peripheral blood eosinophil levels on clinical and immunological features of BP. Peripheral blood eosinophil levels were recently shown to be positively correlated with peripheral blood levels of both IgG and IgE anti-BP180 and with disease severity.^{7,12} Kridin reported that patients with BP and eosinophilia had a higher rate of palmoplantar involvement and extensive disease. In contrast, patients with normal eosinophil counts tended to have atypical clinical features including higher mucosal and head/neck involvement.⁷

Although an inflammatory infiltrate rich in eosinophil is a well-known feature of BP, the relation between tissue eosinophil levels and the presentation of BP is yet to be established. Izumi et al. showed that patients who presented with an inflammatory phenotype with erythema and urticarial plaques had an increased seropositivity of BP180 NC16A IgG and a higher number of infiltrating eosinophils in lesional skin sections.¹³ Moreover, tissue eosinophil levels have been recently correlated with disease severity.⁸

The current study supports the association of peripheral blood eosinophilia and high dermal eosinophil counts with BP severity. Likewise, we found an association between disseminated forms of disease and high titles of BP180 NC16A IgG, which are well-known to parallel BP activity. In contrast, we found a significant association between mucosal involvement and lower dermal eosinophil levels in concordance with a recent study reporting a higher prevalence of mucosal involvement in patients with a cell-poor inflammatory infiltrate.¹⁴

We observed that patients with high peripheral blood eosinophil levels have an increased risk of requiring inpatient care and relapsing after successful disease control. In contrast, dermal infiltrate eosinophil levels were not associated with treatment outcomes. Therefore, high peripheral blood eosinophil levels may signify a more recalcitrant clinical presentation necessitating more aggressive management. Notably, almost half of the patients who experienced a relapse had high or very high peripheral blood eosinophil levels at the time of relapse. Moreover, we found a strong and significant correlation between peripheral blood eosinophil counts at baseline and at relapse. Our present results suggest that peripheral blood eosinophil levels may be a biomarker of BP relapse. Most cases of clinical relapse of BP occur in the first six months of follow-up. Therefore, monitoring peripheral blood eosinophil counts in this period

Table 2 Relationship between peripheral blood and dermal infiltrate eosinophil counts and demographic, clinical, and immunological characteristics of BP patients.

	Peripheral blood eosinophil count		P-value	Dermal infiltrate eosinophil count		P-value
	Normal (n = 96)	High or very high (n = 137)		No-low (n = 83)	Intermediate-high (n = 150)	
<i>Age at diagnosis, years</i>						
Mean (SD)	77.2 (9.0)	79.7 (9.8)	0.090	78.9 (9.5)	79.8 (8.8)	0.478
<i>Sex, n (%)</i>						
Male	42 (43.8)	81 (59.1)	0.054	42 (50.6)	81 (54.0)	0.579
Female	54 (56.3)	56 (40.9)		41 (49.4)	69 (46.0)	
<i>Comorbidities, n (%)</i>						
Type 2 diabetes mellitus	54 (56.3)	59 (43.1)	0.039	53 (63.9)	60 (40.0)	0.012
DPP-4 inhibitor-associated BP	39 (40.7)	33 (24.1)	0.008	33 (39.7)	39 (26.0)	0.013
Neurologic disease	38 (39.6)	55 (40.1)	0.987	27 (32.5)	66 (44.0)	0.032
Malignancy	12 (12.5)	21 (15.3)	0.825	15 (18.1)	16 (10.7)	0.162
<i>Distribution of bullous lesions, n (%)</i>						
Upper limbs	79 (82.2)	126 (92.0)	0.141	80 (96.4)	125 (83.3)	0.662
Lower limbs	78 (81.2)	124 (90.5)	0.204	76 (91.6)	126 (84.0)	0.678
Trunk	85 (88.5)	114 (83.2)	0.128	78 (94.0)	121 (80.7)	0.566
Hands and feet	61 (63.5)	108 (78.8)	0.029	58 (69.9)	111 (74.0)	0.082
Head and neck	28 (29.1)	29 (21.1)	0.207	27 (32.5)	30 (20.0)	0.104
Mucosal involvement	19 (19.8)	20 (14.6)	0.185	24 (28.9)	15 (10.0)	0.001
Disseminated presentation (i.e. BSA > 50%)	39 (40.6)	74 (54.0)	0.028	43 (51.8)	70 (46.7)	0.868
<i>Anti-BP180 NC16A IgG ELISA</i>						
Mean (SD); (U/mL)	96.7 (84.4)	164.9 (71.5)	<0.001	119.5 (86.9)	134.2 (84.9)	0.419
<i>Anti-BP230 IgG ELISA</i>						
Mean (SD); (U/mL)	23.0 (15.8)	41.5 (36.2)	0.154	22.7 (22.0)	43.0 (28.7)	0.101
<i>Linear deposits of immunoreactants by direct immunofluorescence, n (%)</i>						
C3	92 (95.8)	134 (97.8)	0.563	79 (95.2)	147 (98.0)	0.794
IgG	78 (81.3)	130 (94.9)	0.223	82 (98.8)	126 (84.0)	0.381
IgA	7 (7.3)	10 (7.3)	0.799	7 (7.3)	10 (7.3)	0.799
IgM	2 (2.1)	3 (2.2)	1.000	2 (2.1)	3 (2.2)	1.000
Isolated C3	8 (8.3)	10 (7.3)	0.656	7 (7.3)	11 (7.3)	1.000
<i>Treatment; n (%)</i>						
Oral prednisone (>0.5 mg/kg/day)	86 (89.6)	133 (97.1)	0.487	81 (97.6)	138 (92.0)	0.987
Tetracycline	46 (47.9)	63 (46.0)	0.632	41 (49.3)	68 (45.3)	0.675
Adjuvant immunosuppressant	29 (30.2)	45 (32.8)	0.742	29 (34.9)	45 (30.0)	0.557
Isolated topical treatment	3 (3.1)	2 (1.5)	0.993	2 (2.4)	3 (2.0)	0.887
<i>Number of treatment lines</i>						
Mean (SD)	2.0 (1.1)	2.0 (1.0)	0.928	1.8 (0.9)	1.9 (1.1)	0.455
Inpatient care; n (%)	29 (30.2)	60 (43.8)	0.025	38 (45.8)	51 (34.0)	0.118
Relapsing; n (%)	18 (18.8)	50 (36.5)	0.020	27 (32.5)	41 (27.3)	0.626

Significant values are shown in bold.

BP: bullous pemphigoid, DPP4: dipeptidyl peptidase-4, ELISA: enzyme-linked immunosorbent assay, n: number, SD: standard deviation.

may allow early detection of relapse, particularly in patients with high baseline peripheral blood eosinophil counts.

Our study disclosed a significant association between the comorbidities of BP patients and both peripheral blood and dermal infiltrate eosinophil levels. Similar to previous studies, diabetic patients treated with DPP-4 inhibitors had lower circulating and dermal infiltrate eosinophil counts.^{15–17} In contrast, we found that BP patients with accompanying neurologic disease likely present high circulating eosinophil levels and an eosinophil-rich inflammatory infiltrate. While the association of neurologic diseases in BP with high levels of peripheral blood eosinophils had been previously reported, its association with high dermal eosinophil levels was only identified in a recently published study.^{8,18}

A pivotal contribution of eosinophils in BP pathogenesis has long been suggested as increasing evidence of its biological actions accumulate. Increased expression of eotaxin-1 and Interleukin (IL) -5, two key mediators in the recruitment and activation of eosinophils, has been demonstrated in peripheral blood and blister fluid of BP patients.^{19,20} Following activation, in the presence of BP autoantibodies, eosinophils directly contribute to BP blister formation. Eosinophils degranulate and release granular proteins, such as eosinophil cationic protein, eosinophil extracellular traps and matrix metalloproteinase that directly contribute to BP antigens degradation and dermal–epidermal junction cleavage.^{10,11}

Eosinophils may be a target for new therapeutics in BP.²¹ In fact, the increasing number of cases of successful treatment of BP with omalizumab supports this strategy.^{22,23} Other potential emerging drugs for BP treatment include bertilimumab, a fully human monoclonal antibody targeting eotaxin-1, and anti-IL5 monoclonal antibodies, such as mepolizumab and benralizumab.^{15,24,25} Determination of peripheral blood and dermal eosinophil levels may have a role in patient selection for these treatments. Although clearly promising, further studies are necessary to evaluate the efficacy and safety of eosinophil-directed therapies in the management of BP.

The main limitation of the current study is its retrospective design. Disease severity evaluation was limited by the lack of data on standardised BPADI index for each patient. Besides, eosinophil tissue levels were evaluated on a single lesional punch biopsy. However, biopsies from all patients were obtained from the most inflammatory skin lesion and count was performed by three Dermatopathologists. Selection of more severe and recalcitrant cases may have arisen as the study was carried out in a tertiary referral centre setting. Nevertheless, our study sheds light on a topic that has not been fully investigated so far. Moreover, it provides novel insight regarding the relation between BP clinical and immunological characteristics and peripheral blood and dermal infiltrate eosinophil levels.

In conclusion, circulating and cutaneous eosinophils levels may be useful biomarkers for disease activity and treatment outcomes in BP. Monitoring peripheral blood eosinophil counts may allow early detection of relapse. In addition, inhibition of eosinophil activity may be a strategy for new therapeutics in BP.

Conflict of interests

The authors declare that they have no conflict of interest.

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