Plasma Levels of D-dimer and Fibrin Degradation Products Correlate with Bullous Pemphigoid Severity: a Cross-sectional Study

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Research Article

Keywords: D-dimer, fibrinogen/fibrin degradation products, bullous pemphigoid, severity

Posted Date: May 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-523708/v1

Abstract

Background

Bullous pemphigoid (BP), the most frequent blistering dermatosis in the elderly, is associated with increased mortality. The severity of BP can be assessed by detecting the anti-BP180 immunoglobulin G (IgG) titer, but the lab test is not available in many community clinics. BP patients are usually in a hypercoagulable state with increased levels of D-dimer and fibrin degradation products (FDPs).

Objectives

To evaluate the use of D-dimer and FDPs in assessing BP severity.

Methods

We compared the levels of plasma D-dimer, FDPs, eosinophils, and anti-BP180 IgG titer between 48 BP patients and 33 Herpes zoster (HZ) patients (control group). Correlational analyses were conducted to determine the relationships between the lab values and BP.

Results

The plasma D-dimer and FDP levels were higher in BP patients than in HZ controls (D-dimer: 3297 ±2517 μg/L vs. 569.70 ±412.40 μg/L; FDP: 9.74 ±5.88 mg/L vs. 2.02 ±1.69 mg/L, respectively, P<0.0001). Significant positive correlations were found between D-dimer/FDP levels and BP severity markers (anti-BP180 IgG titer [D-dimer: r=0.3928, P=0.0058; FDP: r=0.4379, P=0.0019] and eosinophil counts [D-dimer: r=0.3625, P=0.0013; FDP: r=0.2880, P=0.0472]) in BP patients. We also found an association between FDP and urticaria/erythema lesions (r=0.3016, P=0.0372), but no other BPDAI components. In 19 BP patients with complete remission after systemic glucocorticoid treatment, D-dimer and FDP levels decreased post-therapy (D-dimer: 5559 ±7492μg/L vs. 1738 ±1478 μg/L; P<0.0001; FDP: 11.20 ±5.88 mg/L vs. 5.13 ±3.44 mg/L; P=0.0003), where as they did not in BP patients with treatment resistant.

Conclusion

Plasma D-dimer and FDP are convenient markers to evaluate BP severity.

Introduction

Bullous pemphigoid is a rare autoimmune subepidermal blistering disorder with an annual incidence estimation of 2.4–23 cases per million in the general population[1]. The bullae form due to autoantibodies against the structural components of hemidesmosomes (including BP180) at the dermal-epidermal junction and eosinophil infiltration into the superficial dermis[2]. BP affects individuals worldwide, especially patients over 70 years of age. Timely assessment and treatment decreases the risk of morbidity and mortality associated with BP. Dermatologists cannot accurately assess BP severity based

on skin examination alone yet they need to know the severity in order to prescribe effective treatment[3,4]. BP severity can be evaluated via anti-BP180 immunoglobulin G (IgG) titers[5]. However, given BP's rarity, many community clinics in China do not have access to this test. There remains an urgent need to identify readily accessible tests that can assess BP severity.

D-dimer is a type of FDP whose levels can reflect the degree of coagulation activation and fibrin formation[6,7]. Increased plasma D-dimer and FDPs have been widely used to evaluate disease severity in a wide range of conditions, including COVID-19, Crohn's disease, and pulmonary embolism[8–11]. Since BP patients are often in a hypercoagulable state with elevated levels D-dimer and FDP[12], we investigated whether plasma D-dimer and FDP levels of BP patients prior to treatment could be used to assess BP severity.

Materials And Methods

Patients

We assessed plasma D-dimer and FDP in 48 BP patients and 33 age- and gender-matched HZ patients. We used HZ patients as the control group to exclude advanced age as a confounder, since plasma D-dimer and FDP levels tend to rise with age. The cross-sectional study was conducted at the Second Affiliated Hospital of Xi'an Jiaotong University (Shaanxi, China) after obtaining approval from its ethics committee. BP was diagnosed based on clinical, histopathological, serological, and immunofluorescent features (shown in Fig. S1). The study involved BP patients older than 18 years of age and without systemic glucocorticoid treatment prior to admission (Table 1). We excluded BP patients with vascular diseases and abnormal renal or liver function, which are known to affect the plasma D-dimer and FDP levels.

Table 1

Inclusion and exclusion criteria for a cross-sectional study of BP patients

Inclusion criteria	Exclusion criteria
· Age > 18 years old · Availability of recent coagulation test results · Clinically significant subepidermal blisters ≥ 5 mm in diameter (defined as cutaneous blisters or ruptured blisters with a flexible roof covering a moist base) · Dermal-epidermal separation surrounded by eosinophils on pathology · Anti-BP180 ≥ 9 IU/mL · Immunofluorescence showed IgG and/ or C3 deposit · No systemic glucocorticoid treatment before admission	A history of · Vascular disease (e.g., deep venous thrombosis and aortic dissection) · Anticoagulation therapy · Abnormal liver and kidney function · Cardiovascular disease · Diabetes mellitus · Cerebrovascular disease · Acute or chronic inflammatory disease · Malignancy with or without treatment

Twenty-eight patients post systemic glucocorticoid treatment (methylprednisolone 40-80 mg/day at progressively tapered doses) were followed up. The corticosteroid treatment led to complete clinical remission (defined as the absence of new BP lesions for a minimum of four weeks with complete healing of the prior lesions), partial remission (defined as incomplete healing of the prior lesions), or non-remission (absence of healing and growth of ≥ 10 new BP lesions per day). The patients were given low-dose corticosteroids (≤ 30 mg/day) when re-evaluating the levels of D-dimer and FDP (seven to ten days after the beginning of corticosteroid treatment),

All patients gave informed consent. The trial registration number was ChiCTR1800017560.

Biochemical detection

Whole blood was collected in lithium heparin tubes (BD, USA) from all the patients before systemic glucocorticoid therapy and after in some. The eosinophil cell count was obtained from the whole blood. Plasma was separated according to standard protocol and D-dimer and FDP values were analyzed (Sysmex CA-7000, Japan).

Serum was obtained from blood and anti-BP180 IgG titer (A MESACUP BP180-ELISA kit, MBL, Nagoya, Japan) was detected according to the manufacturer's instruction. A positive was defined as ≥ 9 IU/mL.

Severity assessment

The BPDAI was used to estimate the severity, including three parameters that constitute the total BPDAI activity (erosions/blisters, urticaria/erythema, and mucosal blisters/erosions) and total BPDAI damage (pigmentation) 13. The BPDAI scores range from 0 - 360 for total BPDAI activity (maximum score of 120 for each activity parameter) and 0 - 12 score for damage, with higher scores indicating greater disease activity or damage. We separated the BPDAI activity components in order to analyze the correlation between the lesion types and the coagulation markers.

Statistical analysis

We used GraphPad Prism version 8.0.1 software (GraphPad Software, La Jolla, CA) for all statistical analyses. Continuous data were expressed as mean ± SD. Groups were compared by the Fisher's exact test, Chi-square test, or unpaired t-test. Correlations between two parameters were analyzed using Pearson's correlation test. Differences were considered statistically significant at $P < 0.05$. "*", "**", and "***" represented $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Results

Descriptive Analysis of the Cohort

A total of 48 BP patients (29 men [63.6%] and 19 women [36.4%]) and 33 HZ patients were enrolled. There was no statistically significant difference in sex or age between BP patients and HZ controls ($P > 0.05$, Table 2). The average BP duration was 15.51 ± 45.73 months (range 0.2–304 months). Clinical information can be found in Table S1.

Table 2

Demographic characters of patients with HZ and BP

Groups	HZ	BP	*P*-value
No.	33	48	
Age	69.03 ±8.87	71.17 ±8.62	0.2820
Gender			0.4966
Male	17	29	
Female	16	19	
D-dimer (μg/L)	569.70 ±412.40	3297 ±2517	< 0.0001
FDP (mg/L)	2.02 ±1.69	9.74 ±5.88	< 0.0001
Eosinophils ($\times 10^9$/L)	0.11 ±0.12	1.16 ±1.83	0.0013

Elevated D-dimer, FDP and eosinophil count in BP

Compared to the age- and gender-matched HZ patients, BP patients had elevated plasma D-dimer and FDP levels (3297 ±2517 μg/L vs. 569.70 ±412.40 μg/L; 9.74 ±5.88 mg/L vs. 2.02 ±1.69 mg/L, respectively, P<0.0001, Table 2 and Fig. 1A, B). BP patients had higher eosinophil counts than HZ patients (1.16 ±1.83×10^9/L vs. 0.11 ±0.12×10^9/L, P=0.0013, Table 2 and shown in Fig. 1C).

Anti-BP180 IgG titers correlate with BPDAI

We found that anti-BP180 IgG titer was associated with total BPDAI score (r=0.3251, P=0.0242, Fig. S2A). To determine the relationships between anti-BP180 IgG titer and individual BPDAI components, we found a moderate correlation with erosions/blisters (r=0.3402, P=0.0180), but not urticaria/erythema (r=0.1261, P=0.3931), pigmentation (r=0.2797, P=0.0542), and mucosal damage (r=-0.0833, P=0.5734, shown in Fig. S2B).

D-Dimer, FDP, and Eosinophil correlation with anti-BP180 IgG titers and BPDAI

The levels of plasma D-dimer (r=0.3928, P=0.0058) and FDP (r=0.4379, P=0.0019) were positively correlated with the anti-BP180 IgG titer (shown in Fig. 2A). We did not identify correlations between the coagulation markers and individual BPDAI components except for a mild correlation between FDP and urticaria/erythema (r=0.3016, P=0.0372, shown in Fig. 2B, C).

Given eosinophils' role in BP pathogenesis 14,15, we evaluated eosinophil counts in addition to D-dimer and FDP. We detected a correlation between blood eosinophils and anti-BP180 IgG titer (r=0.3621, P=0.0114, shown in Fig. 3A), as well as plasma D-dimer (r=0.3625, P=0.0013) and FDP (r=0.2880, P=0.0472) levels (shown in Fig. 3B). We also found that eosinophil counts were associated with urticaria/erythema lesions (r=0.5071, P=0.0002, shown in Fig. 3C), but not other lesions.

Treatment response paralleled decreased D-dimer and FDP levels

We measured the plasma D-dimer and FDP levels before and after therapy in 28 BP patients. Among them, 19 patients with complete remission after therapy showed a marked reduction in plasma D-dimer levels (5559 ±7492μg/L vs. 1738 ±1478 μg/L; P<0.0001, shown in Fig. 4A) and FDP levels (11.20 ±5.88 mg/L vs. 5.13 ±3.44 mg/L; P=0.0003, shown in Fig. 4B); nine patients with treatment-resistance had increased plasma D-dimer levels (2780 ±3588 μg/L vs. 4503 ±4032 μg/L, shown in Fig. 4C; P=0.3523) and FDP levels (9.38 ±8.71 mg/L vs. 15.22 ±9.64 mg/L; P=0.1968, shown in Fig. 4D), though the differences were not statistically significant.

Discussion

Identifying accessible tests that can assess BP severity in community clinics lacking anti-BP180 IgG titer tests remain an urgent need. We report a positive association between two coagulation markers (plasma D-dimer and FDP) and known markers of BP severity including BPDAI, anti-BP180 IgG, and eosinophil counts. This association suggests that the coagulation markers can be used to evaluate BP severity. Our study also leads to the belief that anticoagulation may be helpful for bullous pemphigoid as adjuvant therapy [13].

BP's inflammatory lesions lead to activation of the coagulation cascade, resulting in elevated plasma D-dimer and FDP levels[14]. It is no surprise then that D-dimer/FDP levels were increased in treat resistant patients but decreased in treatment responsive patients. Interestingly, we found that FDP levels is associated with urticaria/erythema in BP, which may occur for several reasons: (1) FDP can increase the vascular permeability and thereby induce wheals[15]; (2) thrombin-dependent activation of mast cells [16]; (3) the urticaria/erythema associated with anti-BP180 IgE autoantibodies may increase the FDP level [17]. The failed association between D-dimer and BP lesions suggests that the larger fibrin degradation products contribute to lesion formation.

Eosinophils are critical to BP pathogenesis. We have demonstrated increased eosinophil counts in BP, similar to other studies [18,19], and found a correlation with BP severity. The positive correlation between eosinophil counts and D-dimer/FDP levels may be due to eosinophils' role as major intravascular storage locations for tissue factor (TF)[22], an initial factor of the extrinsic coagulation pathway. TF facilitates the early trans-endothelial migration of eosinophils[22], which can directly damage endothelial integrity.

Eosinophils can release cationic eosinophilic granular proteins and alter the cutaneous microcirculation[23, 24], which may contribute to the correlation we observed between eosinophils and urticaria/erythema lesions.

Autoantibodies to coagulation factors could modulate their activity[23]. Given that anti-BP180 autoantibody levels correlated with D-dimer/FDP levels, it may suggest that anti-BP180 IgG antibodies can activate coagulation factors. Anti-BP180 autoantibodies, especially IgG type, have been found to negate, alter, or promote the rapid clearance of clotting factors [23,24]. The anti-BP180 autoantibody may also mediate the formation of neutrophil extracellular traps (NETs) and induce a hypercoagulable state, previously seen in COVID-19 patients[25–27]. The precise mechanism on autoantibodies and hypercoagulation in BP requires further study.

Limitations of the study include a limited clinical recruitment site at an inpatient department of a single hospital, which may introduce selection bias. Additional medical centers and BP outpatients (usually expressing mild symptoms) should be considered in future studies. The selection criteria for the control group were not optimal, because blisters in HZ were usually limited and the magnitude of blisters can be mild compared to that of BP patients. The comparison of BP to HZ patients can potentially involve a risk of obtaining false-positives in the statistical analyses. A larger sample size is needed to accurately assess the markers to confirm their clinical significance. In addition to anti-BP180 IgG, anti-BP180 IgE and anti-BP230 are also associated with BP severity and their relationships with D-dimer/FDP levels should also be evaluated.

Conclusion

In BP patients, plasma D-dimer and FDP levels are correlated with current BP severity markers including anti-BP180 IgG titers, BPDAI, and eosinophil counts. The two coagulation markers may be potential means to evaluate BP severity.

Declarations

Acknowledgement

We thank patients in this manuscript who signed the written informed consent and allowed us to publish their clinical data.

Statement of Ethics

This clinical trial was approved by the Ethics Committee of the Second Affiliated Hospital, Xi'an Jiaotong University. All participants provided informed consent and signed the informed consent form. Written informed consent for publication of the patients' clinical details and/or clinical images was obtained

from the patients/guardians of the patients. All experiments were implemented accordant with relevant guidelines and regulations.

Conflict of Interest Statement

The authors declare no competing interests.

Funding Sources

The National Natural Science Foundation of China (Project No. 81803137), the Free Exploration Plan of the Second Affiliated Hospital of Xi'an Jiaotong University (No. RC(XM)201807), and the Innovation Capability Support Plan of Shaanxi Province (No. 2019TD- 034). The funders contributed to the design of the study, to the collection, analysis and interpretation of data, and to the potential publication fee.

Author Contributions

YL and YX designed the experiment. SW, ML, XP, and MF handled the blood specimens and did the clinical test. SW, ML, ZZ, XP, LL, CC, and YL drafted the manuscript. YX, ZZ, CC, LL, and YL revised the draft. All the authors reviewed the manuscript and approved the final version.

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Figures

Figure 1

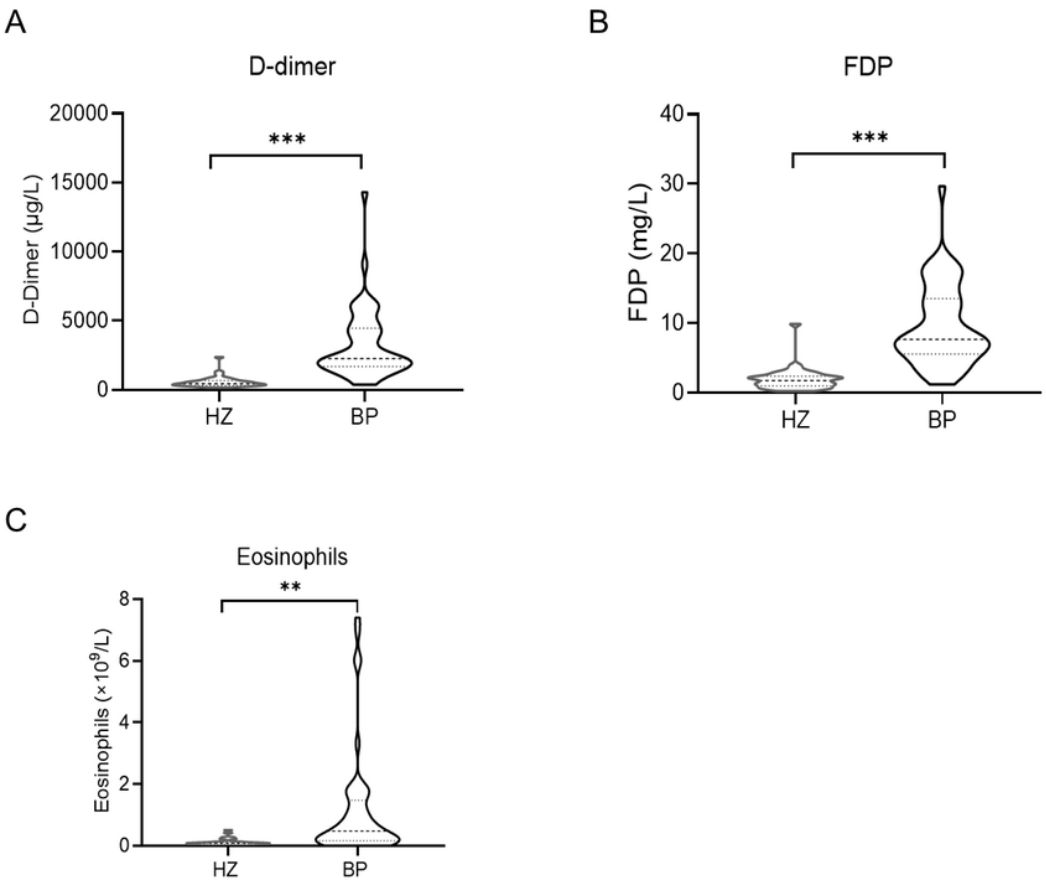
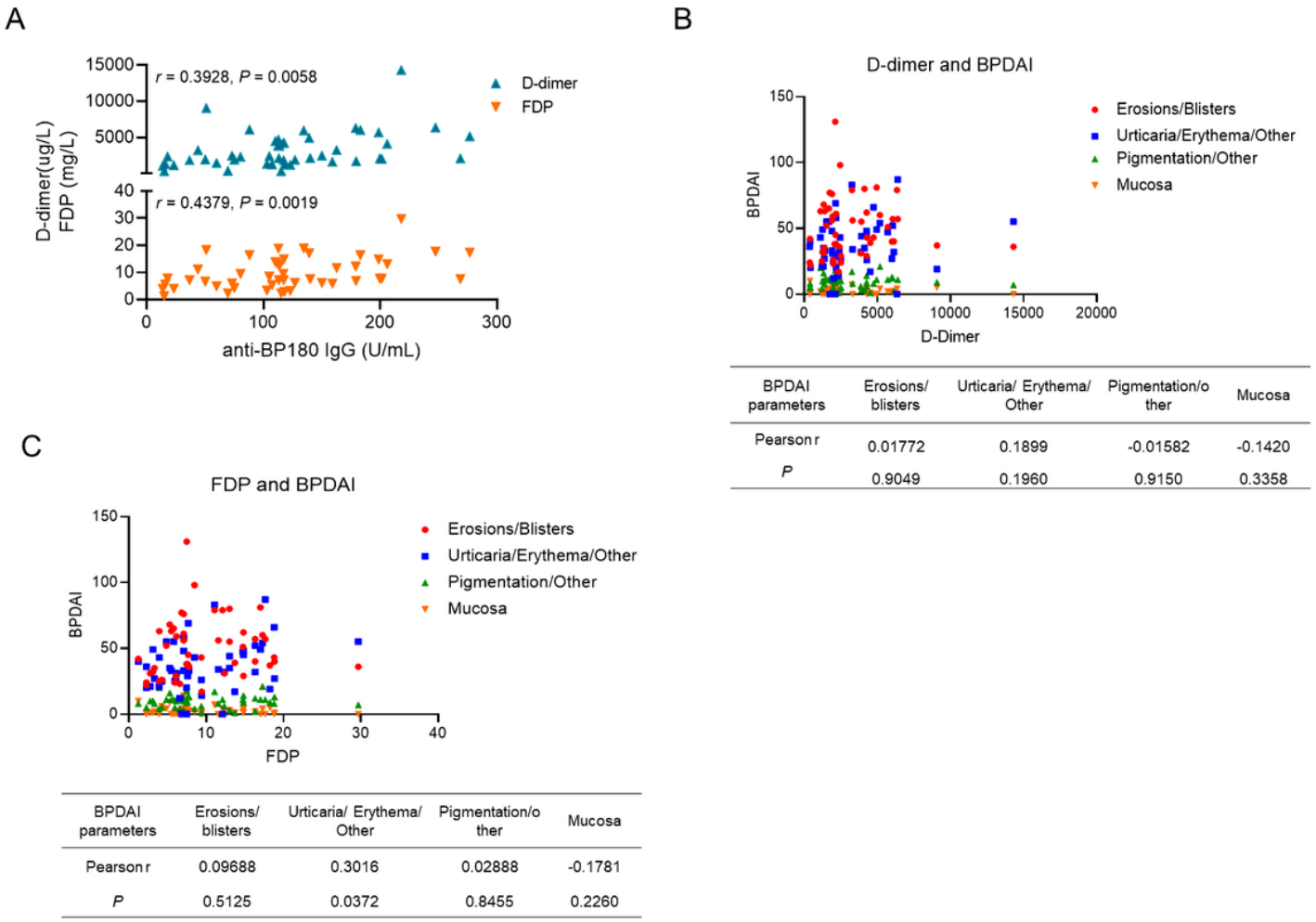


Figure 1

The levels of D-Dimer, FDP, and eosinophils in 48 BP patients and 33 HZ controls. A & B. D-dimer and FDP levels were increased in BP patients compared to HZ controls; C. Peripheral blood eosinophil counts in BP patients were higher than those in HZ controls.

Figure 2



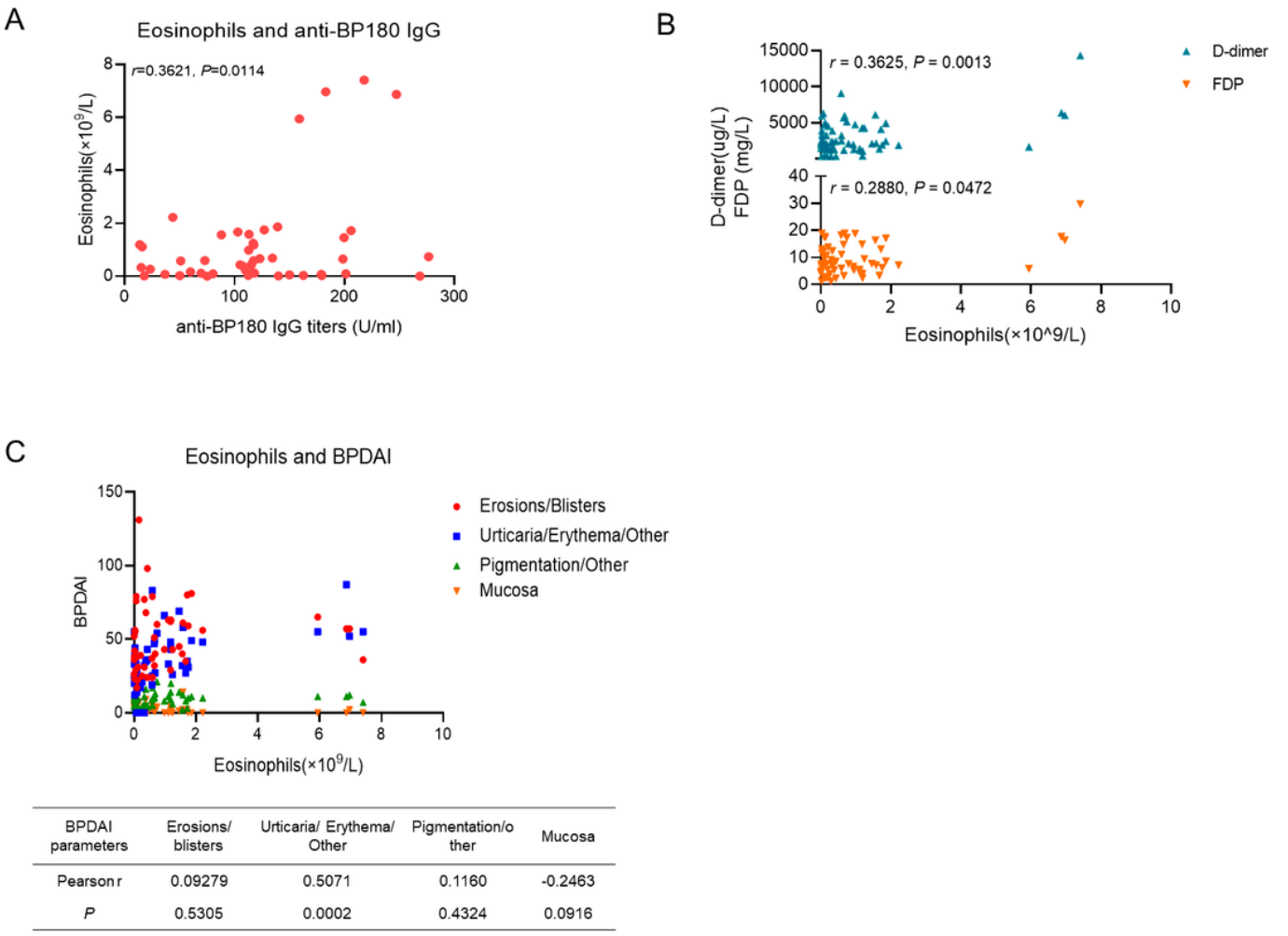
BPDAI parameters	Erosions/ blisters	Urticaria/ Erythema/ Other	Pigmentation/o ther	Mucosa
Pearson r	0.01772	0.1899	-0.01582	-0.1420
P	0.9049	0.1960	0.9150	0.3358

BPDAI parameters	Erosions/ blisters	Urticaria/ Erythema/ Other	Pigmentation/o ther	Mucosa
Pearson r	0.09688	0.3016	0.02888	-0.1781
P	0.5125	0.0372	0.8455	0.2260

Figure 2

The correlation among D-Dimer, FDP, anti-BP180 IgG titers, and BPDAI. A. The levels of D-dimer and FDP correlate with anti-BP180 IgG titers; B & C. The relationship between BPDAI and the levels of D-dimer and FDP.

Figure 3



BPDAI parameters	Erosions/ blisters	Urticaria/ Erythema/ Other	Pigmentation/o ther	Mucosa
Pearson r	0.09279	0.5071	0.1160	-0.2463
P	0.5305	0.0002	0.4324	0.0916

Figure 3

The correlation among eosinophils, D-Dimer, FDP, anti-BP180 IgG titers, and BPDAI. A. The correlation between eosinophils and anti-BP180 IgG; B. The relation of eosinophils and levels of D-dimer and FDP; C. The relation between BPDAI and eosinophil counts.

Figure 4

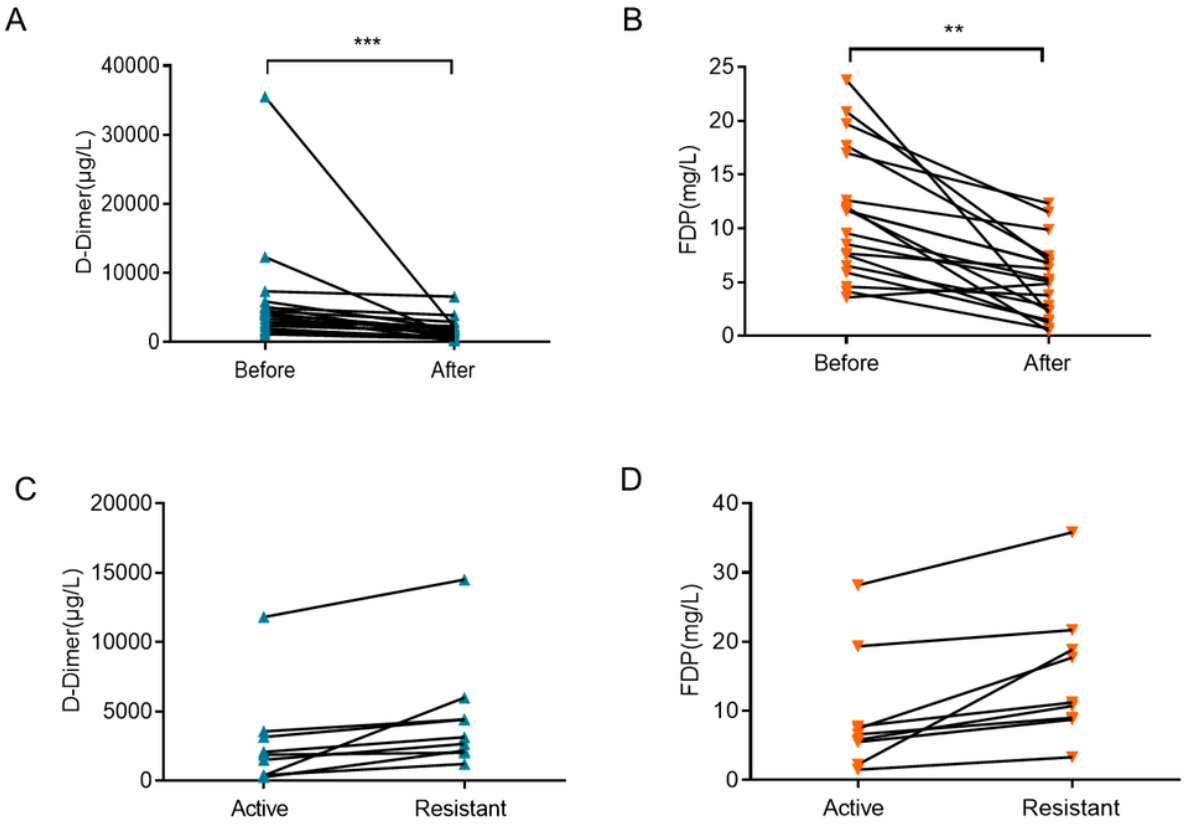


Figure 4

D-dimer and FDP levels in BP patients with treatment-effective and -resistant BP. Plasma D-dimer (A) and FDP (B) levels in 19 patients with treatment-effective BP were evaluated before and after immunosuppressive therapy. Marked reductions in both coagulation markers were observed after complete remission. Plasma D-dimer (C) and FDP (D) levels in nine patients with treatment-resistant BP were evaluated before and after immunosuppressive therapy. A slight elevation in both was observed without relief.

Supplementary Files

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- Fig.S1.tif
- Fig.S2.tif
- TableS1ClinicalinformationofBPandHZpatients.xlsx