

Serum levels of D-dimer and fibrinogen/fibrin degradation products correlate with BP severity

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Abstract

Background

Bullous pemphigoid (BP) is the most common blistering dermatosis with increasing mortality. Currently, the severity of BP can be assessed by the detection of anti-BP180 immunoglobulin G (IgG) titer, but it is absent in many grassroots clinics. BP patients are usually in a hypercoagulable state, and the levels of D-dimer and fibrin degradation products (FDPs) are elevated. Therefore, we aim to evaluate the use of D-dimer and FDPs in the assessment of BP severity.

Methods

This study included 33 BP and 33 Herpes zoster (HZ) patients, with the HZ patients serving as a control. The levels of plasma D-dimer and FDPs as along with eosinophil counts were recorded during a routine screening examination. Anti-BP180 IgG titer was tested by ELISA. BP lesion area was evaluated on admission. Correlational analyses were carried out between these indexes.

Results

The plasma D-dimer and FDP levels were higher in BP patients than in HZ patients. A significant positive correlation was found between the lesion area and both D-dimer and FDP levels in BP patients. There was also a positive association between anti-BP180 IgG and D-dimer, and between anti-BP180 IgG and FDP.

Conclusions

Plasma D-dimer and FDP may be convenient markers to evaluate the severity of BP.

Background

Bullous pemphigoid, a common autoimmune subepidermal blistering disorders. It is characterized by the presence of circulating autoantibodies against the structural components of hemidesmosomes (including BP180, also known as collagen XVII) and eosinophil infiltration in the superficial dermis, which together cause bullae formation at the dermal-epidermal junction [1]. The disease mainly affects individuals over 70 years of age and is found worldwide in all populations [2]. The morbidity and mortality of BP increase with age because of disease-specific factors; therefore, timely assessment and proper treatment are necessary. Currently, BP severity can be evaluated through the test of anti-BP180 immunoglobulin G (IgG) titers [3]. However, because of the very few BP patients, the facility to detect anti-BP180 antibodies is not available in many grassroots clinics, especially in China. As a result, it is difficult for dermatologists to evaluate changes in patients' conditions and prescribe therapy. Hence, there is an urgent need to assess the BP severity by routine tests.

The hypercoagulable state is characterized by elevated D-dimer and fibrin degradation products (FDPs). Increased D-dimer and FDP levels have been reported in many diseases, including deep venous

thrombosis, disseminated intravascular coagulation, aortic dissection, and Crohn's disease [4, 5]. BP patients are also usually in a hypercoagulable state with elevated levels of several coagulation markers, such as D-dimer and prothrombin fragment F1 + 2 [6]. The measurement of D-dimer and FDPs has been widely used to diagnose diseases and evaluate disease severity [7, 8]. Since BP is characterized by the hypercoagulable state, we considered that D-dimer and FDPs may be useful in assessing BP severity. Moreover, the tests of D-dimer and FDPs are cheaper and more readily available than the test of anti-BP180 IgG titers. Therefore, it is important to explore whether D-dimer and FDP can be used to assess BP severity.

With this background, we investigated the possible correlation of D-dimer and FDP with BP. We did a retrospective analysis to evaluate whether D-dimer and FDP levels in the plasma of BP patients (before any treatment) could be used as markers to evaluate the severity of BP.

Method

Study population and definition of diseases

A total of 66 patients were included in the study (hospitalized in the Second Affiliated Hospital of Xi'an Jiaotong University from October 1st, 2018 to December 31st, 2019), including 33 patients with BP and 33 patients (age- and gender-matched) with HZ as controls. BP was diagnosed based on clinical manifestations; and histopathological, serological, and immunofluorescent features (Figure S1). The selection criteria included age more than 18 years; clinically significant subepidermal blisters defined as cutaneous blisters at least 5 mm in diameter or ruptured blisters with a flexible (not dry) roof covering a moist base; dermal-epidermal separation surrounded by eosinophils in pathology; and with no systemic glucocorticoid treatment before admission. Patients with a history of venous thrombosis, anticoagulation therapy, cardiovascular disease, diabetes mellitus, cerebrovascular disease, acute or chronic inflammatory disease, malignancy, or anticancer treatment were excluded.

The protocol was approved by the ethics committee of the Second Affiliated Hospital, Xi'an Jiaotong University. Informed consent was obtained from all patients on admission, including patients whose serum samples were collected. The trial registration number was ChiCTR1800017560.

Biochemical Detection

The eosinophil cell count (differential and coagulation) was performed in all patients on admission. Erythrocyte sedimentation rate (ESR) was assessed and used as both continuous and dichotomous variables. For the dichotomous variable, we set a cut-off value of 30 mm/h, as this was proven to represent a risk factor for fatal outcome in patients with BP[9].

Whole blood was collected into lithium heparin tubes (BD, USA) from a few patients before corticosteroid therapy. To separate serum, whole blood was mixed with trisodium citrate dihydrate (0.11 mol/L) in a 9:1

ratio in a serum separator tube and centrifuged at 3000 × rpm for 10 min. D-dimer and FDP values were analyzed from the serum by CA-7000 (Sysmex, Japan).

Serum was centrifuged at 1000 × rpm, 4 °C for 5 min and stored at – 80 °C for anti-BP180 IgG detection. A MESACUP BP180-ELISA kit (MBL, Nagoya, Japan) was used for analyzing the serum titers of anti-BP180 IgG. Recombinant NC16a protein provided in the kit was used to capture BP180 IgG in serum (1:101). The second reaction was developed using horseradish peroxidase-conjugated goat anti-human IgG secondary antibody (100 ul/well, provided in a pre-diluted form in the kit). The positive value was defined as > 9 IU/mL.

Twenty-nine patients who had completed corticosteroid treatment (methylprednisolone 40–80 mg/day at progressively tapering doses) were studied. The corticosteroid treatment led to complete clinical remission (defined as the absence of any new BP lesions for a minimum of four weeks with complete healing of the previous lesions), partial remission (defined as incomplete healing of the previous lesions), or non-remission (absence of healing, moreover the growth of more than ten new BP lesions every day). During the re-evaluation sampling (7 to ten days after the beginning of corticosteroid treatment), the patients were given low-dose corticosteroids (\leq 30 mg/day).

Severity Assessment

The lesion area was used to estimate BP severity [10]. Lesions included edematous erythema and blistering. Patients were classified into three groups by the percentage of the affected area: <30%, 30–60%, and > 60% of the whole body surface.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 5.0 software (GraphPad Software, La Jolla, CA). Data are summarized and shown as means \pm SD. Correlations between two parameters were analyzed using Pearson's correlation test. The difference between the two groups was examined by chi-square test or unpaired *t*-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

33 BP patients (21 men [63.6%] and 12 women [36.4%]) with hypercoagulation, but without heart disease, diabetes, or abnormal renal and liver functions, were included. HZ data was collected from test records ($n = 33$). There was no statistically significant difference in sex or age between BP patients and HZ control patients (both $P > 0.05$, Table 1). The average disease duration was 11.22 ± 2.24 months (range 0.2–300 months), and the average skin lesion area was 50.94 ± 22.97 (range 10–95).

Table 1
Demographic characters of patients with HZ and BP

Groups	HZ	BP	<i>P</i> -value
No.	33	33	
Age	69.03 ± 8.87	71.67 ± 8.69	0.2269
Gender	17	21	0.4553
Man	16	12	
Woman			
D-dimer (µg/L)	569.70 ± 412.40	2338 ± 2266	< 0.0001
FDP (mg/L)	2.02 ± 1.69	7.81 ± 6.64	< 0.0001
Eosinophils (× 10 ⁹ /L)	0.11 ± 0.12	0.77 ± 1.25	0.0035

Elevated D-dimer, FDP And Eosinophils In BP

Compared to the age- and gender-matched HZ patients, BP patients had elevated plasma D-dimer and FDP levels (2338 ± 2266 µg/L vs. 567.90 ± 412.40 µg/L; 7.81 ± 6.64 mg/L vs. 2.02 ± 1.69 mg/L, $P < 0.0001$, Table 1 and Fig. 1A, B). BP patients also had higher eosinophil counts than did HZ patients (0.77 ± 1.25 × 10⁹/L vs. 0.11 ± 0.12 × 10⁹/L, $P = 0.0035$, Table 1 and Fig. 1C). The increase of D-dimer in BP patients with elevated D-dimer was 93.9% (n = 31) compared to only 6.1% (n = 2) in BP individuals with normal D-dimer. The increase of FDP in BP patients with elevated FDP was 69.7% (n = 23) compared to 30.3% (n = 10) in individuals without elevated FDP. Of the 33 BP patients, 24 patients had elevated eosinophils, whereas nine patients showed reduced eosinophils ($\leq 0.01 \times 10^9$ /L).

D-Dimer and FDP correlated with the BP severity indexes of lesion area and anti-BP180 IgG titers

Lesion area and anti-BP180 IgG titers were used to evaluate disease severity. The anti-BP180 IgG titer strongly correlated with the lesion area ($r = 0.59$, $P = 0.0003$, Fig. 2B). We assessed the correlation of plasma D-dimer and FDP levels with these indexes. The levels of plasma D-dimer and FDP in patients with larger lesion area were significantly higher than those with smaller lesion area (Fig. 2A). The levels of plasma D-dimer ($F = 3.86$, $P = 0.0240$) and FDP ($F = 4.36$, $P = 0.0154$) also positively correlated with the lesion area (Fig. 2B). In addition, the titer of anti-BP180 IgG correlated with levels of plasma D-dimer ($r = 0.50$, $P = 0.0037$), and FDP ($r = 0.50$, $P = 0.0028$) levels (Fig. 2A).

Eosinophils correlated with D-Dimer and FDP levels in BP patients

There was a weak correlation between the titer of anti-BP180 IgG and the number of blood eosinophils ($r = 0.12$, $P = 0.0487$), but the patients with the most blood eosinophils had the highest level of serum anti-BP180 IgG. Plasma D-dimer ($r = 0.18$, $P = 0.0374$) and FDP ($r = 0.13$, $P = 0.0374$) levels also correlated positively with blood eosinophil counts (Table 2). However, blood eosinophil counts did not correlate with the lesion area ($r = 0.06$, $P = 0.1810$, Table 2).

Table 2
The correlation between the serum parameters and eosinophils

Groups	BP180-IgG	D-Dimer	FDP	Lesion area
Correlation coefficient	0.12	0.18	0.13	0.06
P-value	0.0487	0.0374	0.0374	0.1810

High Coagulation Activity Correlated With Increased Systemic Inflammation

We found a statistically significant correlation between the ESR levels as a continuous variable (mm/h) and the anti-BP180 IgG titers ($P = 0.0022$, Figure S2A). But, we detected no statistically significant correlation between ESR levels and the lesion area ($P = 0.3630$, Figure S2B). The ESR levels in different lesion area groups also showed no differences (Figure S2C).

For the dichotomous analysis, patients with ESR > 30 mm/h showed a significantly higher level of anti-BP180 autoantibodies than patients with ESR < 30 mm/h (193.80 ± 85.21 vs. 99.51 ± 43.10 , $P = 0.0470$, Fig. 3A). Patients with ESR > 30 mm/h also showed a higher D-dimer (5179 ± 8041 $\mu\text{g/L}$) value than patients with ESR < 30 mm/h (2792 ± 1992 $\mu\text{g/L}$, $P = 0.0093$, Fig. 3B). However, the levels of FDP were not significantly increased in patients with ESR > 30 mm/h compared with patients with ESR < 30 mm/h ($P = 0.2861$; Fig. 3C).

Treatment Response Paralleled Decreased D-dimer And FDP Levels

We further performed a longitudinal analysis of the plasma D-dimer and FDP levels before and after therapy in 29 patients with BP. Among them, 20 patients with complete remission showed a marked reduction in plasma D-dimer levels (5559 ± 1675 $\mu\text{g/L}$ vs. 1738 ± 330.4 $\mu\text{g/L}$; $P < 0.0001$, Fig. 4A) and FDP levels (12.99 ± 2.48 mg/L vs. 5.20 ± 0.79 mg/L; $P = 0.0095$, Fig. 4B) after therapy; nine patients with treatment-resistance showed an increase of plasma D-dimer levels (2780 ± 1196 $\mu\text{g/L}$ vs. 4503 ± 1344 $\mu\text{g/L}$, Fig. 4C; $P = 0.3523$) and FDP levels (10.52 ± 3.03 mg/L vs. 14.08 ± 3.31 mg/L; $P = 0.1968$, Fig. 4D), but the differences were not significant.

Discussion

In this retrospective study, we found increased levels of coagulation activation markers in the plasma of BP patients, after excluding BP patients with cardiovascular disease, or abnormal renal or liver function, which are known to affect the D-dimer and FDP levels. We also detected a positive correlation between D-dimer/FDP levels and BP severity indexes, suggesting the biomarker function of D-dimer and FDP in evaluating BP severity. This would especially be useful in hospitals that do not have access to anti-BP180 IgG tests.

BP mainly affects elderly individuals. During the aging process, inflammation and activation of blood coagulation can be enhanced in BP patients [11]. BP patients can also produce factor V inhibitor, leading to bleeding [12]. All these studies support the correlation between BP and activated coagulation cascade. Our findings revealed that the coagulation cascade was activated in BP and correlated with disease severity and eosinophilia, which are in accordance with a study that estimated blood coagulation in 20 BP patients [13]. The most important clinical consequence of the hypercoagulable state in BP is an increased thrombotic risk, which may account for the cardiovascular event in BP patients. The observation that thrombotic complications occur more frequently in BP patients further supports this viewpoint [14]. Our study revealed elevated plasma levels of D-dimer and FDP in BP patients, further verifying the hypercoagulable state.

FDP has been reported to be involved in cardiovascular diseases. Previous studies have found that FDP participates in the progression of atherosclerosis and thrombus. FDP is also a valuable diagnostic biomarker for patent-type acute aortic dissection patients and thrombosed-type acute aortic dissection patients [15]. D-dimer is a type of FDP which can reflect the degree of coagulation activation and fibrin formation [16]. It was introduced as a diagnostic aid for thrombotic diseases such as pulmonary embolus and deep venous thrombosis [17]. Increased D-dimer and FDP have been reported in many diseases, including Kawasaki disease, aortic dissection, and stroke [17, 7]. A strong correlation between FDP and D-dimer was also reported in many diseases [15, 4, 7]. Here, we also found a similar correlation between D-dimer and FDP in BP.

Elevated D-dimer and FDP may manifest as a generalized inflammatory disturbance in BP. The inflammatory response can induce the activation of blood coagulation both locally, by amplifying the inflammatory network in lesions, and systemically, by leading to a prothrombotic state [18]. In the inflammatory response in BP, eosinophils play a critical role. Our and other studies revealed increased eosinophil numbers in BP [14], which is correlated with BP disease severity [14]. The correlation between eosinophils and anti-BP180 IgG titer suggested systemic inflammation. A positive correlation between the levels of blood eosinophils and D-dimer or FDP suggested a systemic prothrombotic state. This viewpoint is supported by the observed reduction of both D-dimer and FDP levels in 20 responsive patients after immunosuppressive treatment, and their increase in 9 resistant patients.

Eosinophils are known to be a major intravascular location for tissue factor (TF) [19], an initial factor of the extrinsic coagulation pathway. TF can specifically facilitate the early transendothelial migration of

eosinophils [19]. In addition, eosinophils can directly damage endothelial integrity by releasing eosinophil granule proteins (EPO) and altering the microcirculation [20]. Eosinophils can also release cationic eosinophilic granular proteins, such as eosinophilic cationic protein (ECP) and major basic protein (MBP), which can neutralize thrombomodulin (highly anionic) via electrostatic binding, finally leading to the inhibition of the thrombomodulin function [21, 22]. In addition, MBP and EPO can activate platelets and promote thrombosis as well [23]. ECP can neutralize heparin and endogenous heparin sulfate, disturbing the anticoagulant function [24]. Thrombin and activated coagulation factors VII and X are proinflammatory mediators, which can induce the production and release of various interleukins, adhesion molecules, selectins, and growth factors, thereby amplifying the systemic inflammatory network [25].

However, we did not find any correlation between eosinophil number and lesion area, which may be because eosinophil numbers are more closely associated with erythematous lesions [26]. Positive correlations among the levels of both D-dimer and FDP, blood eosinophil counts, and lesion area suggest that D-dimer and FDP could be biological indicators of BP disease severity. This viewpoint is supported by the observed reduction of both D-dimer and FDP levels in 20 responsive patients after immunosuppressive treatment, and their increase in 9 resistant patients.

The link between inflammatory response and blood coagulation is further confirmed by a correlation between ESR marker and the hypercoagulable state. Previous findings have shown that an ESR > 30 mm/h correlates with disease activity, and is a risk factor for lethal outcome in patients with Bullous pemphigoid [9]. We found that patients with ESR > 30 mm/h also had higher anti-BP180 IgG titers. Moreover, ESR had a positive correlation with anti-BP180 IgG titers, indicating the potential of ESR in assessing disease severity. We did not find any significant correlation between continuous or dichotomal ESR and lesion area. This may be due to the subjectivity of the lesion area and the non-specificity of ESR. Additionally, patients with ESR > 30 mm/h showed higher levels of D-dimer and FDP, suggesting that increased systemic inflammation may “disturb” coagulation function.

BP is an autoimmune disease mediated by anti-BP180 autoantibodies. The level of anti-BP180 autoantibodies is correlated with BP severity [27]. Our study confirmed this correlation. The previous study reported that autoantibodies could also modulate the activation of hypercoagulation [28]. Our results showing that anti-BP180 autoantibody levels correlated with levels of D-dimer and FDP implicated that coagulation could be modulated by autoantibody production, which is in accordance with a previous study [29]. The correlation between anti-BP180 antibodies and eosinophil counts also suggested the role of inflammation. The disturbed autoimmunity probably leads to the production of anti-BP180 antibodies, which then triggers inflammation and promotes the chemotaxis of eosinophils. Eosinophils not only amplify inflammation but also damage blood vessel endothelium, causing a hypercoagulable state in BP.

Our study had a few limitations. Firstly, as the study was conducted in the in-patient department of only one hospital, there may have been choice bias. Outpatients with BP (usually expressing mild symptoms) should be considered in future studies. The selection criteria for the control group were not optimal, as

blisters in HZ are usually very limited, and the magnitude of blisters can be milder than that of BP patients. Secondly, based on the selection criteria, only a few patients were included in our study. Therefore, there was a risk of obtaining false-positive results in statistical analyses. Larger sample size and longer collection time are needed to accurately assess the markers to confirm their clinical significance. Thirdly, although convenient, evaluation of the lesion area can be rough, and cannot distinguish among erythema, blistering, and erosion. BPDAl could be a more precise indicator than lesion area of disease severity and could help determine the correlations between lesion types and eosinophils, D-dimer or FDP.

Conclusions

Overall, our study reveals that BP patients are in a hypercoagulable state, presenting as increased plasma D-dimer and FDP levels. Also, the D-dimer and FDP levels are correlated with anti-BP180 IgG titers, lesion area, and eosinophil counts in BP patients. Detecting plasma D-dimer and FDP levels may be used as a cheaper and easily available method to evaluate the severity of BP.

List Of Abbreviations

BP, bullous pemphigoid; D-dimer, IgG, immunoglobulin G; FDP, fibrin degradation products; HZ, Herpes zoster; ESR, Erythrocyte sedimentation rate; EPO, eosinophil granule proteins; ECP, eosinophilic cationic protein; MBP, major basic protein.

Declarations

Ethics approval and consent to participate

This clinical trial was approved by the Ethics Committee of the Second Affiliated Hospital, Xi'an Jiaotong University. All participants were undertaken an informed consent procedure and signed the informed consents.

Consent for Publication

Written informed consent for publication of the patients' clinical details and/or clinical images was obtained from the patients/guardians of the patients.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

Fundings

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Authors' contributions

YL and YX designed the experiment. ML, XW and MF handled the blood specimens and did the clinical test. ML, XW, and SW drafted the manuscript with input/modifications from TX and CC. YX and YL revised the draft. All the authors reviewed the manuscript and approved the final version.

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Figures

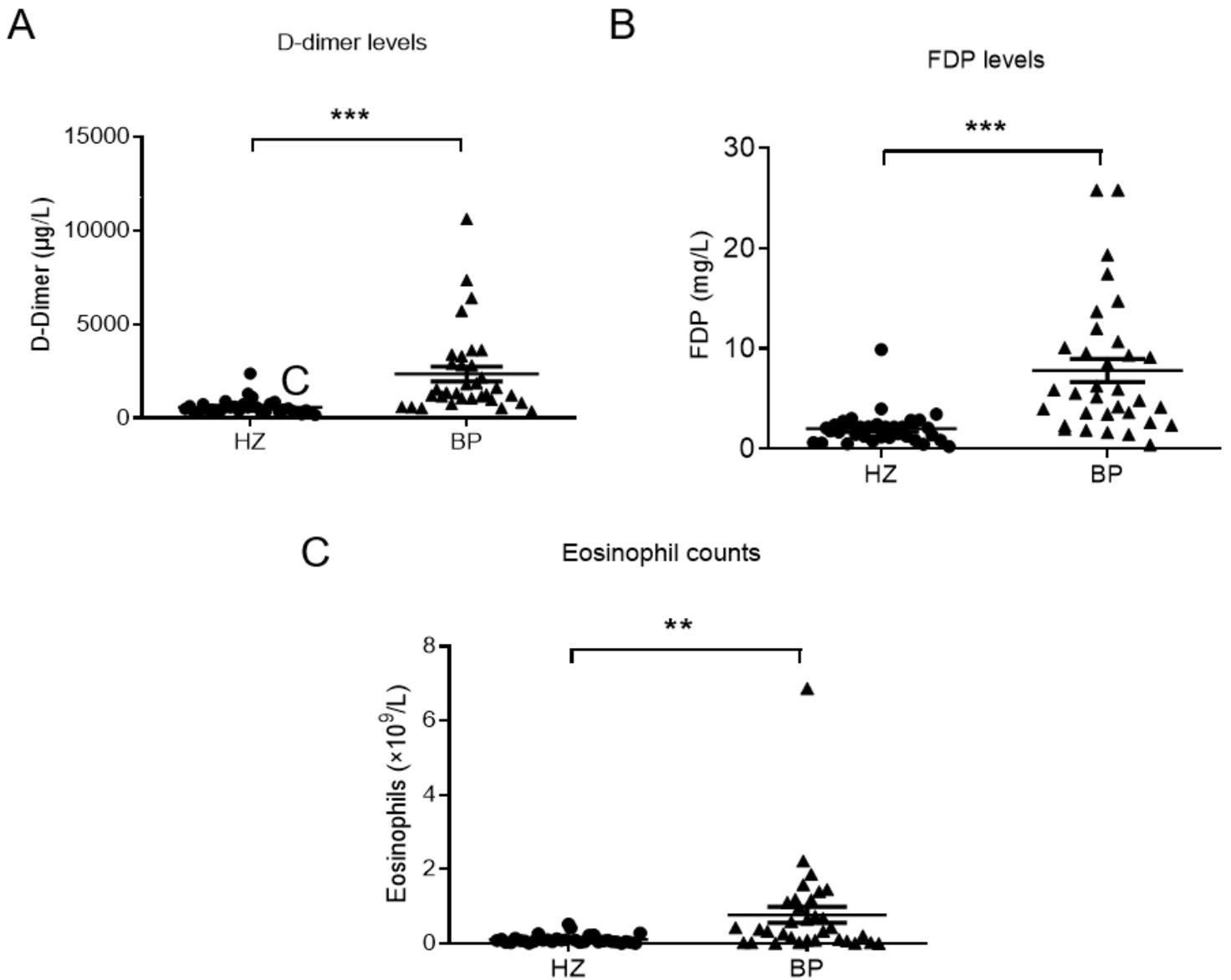


Figure 1

The levels of eosinophils, D-Dimer, and FDP in 33 BP patients and 33 HZ controls. A. The levels of D-dimer were significantly higher in BP patients than that in HZ controls ($P < 0.0001$); B. FDP levels were significantly higher in BP patients than that in HZ controls ($P < 0.0001$); C. Peripheral blood eosinophil counts in BP patients were much higher than those in the HZ control ($P = 0.0035$). BP, bullous pemphigoid; FDP, fibrinogen/fibrin degradation product; HZ, herpes zoster.

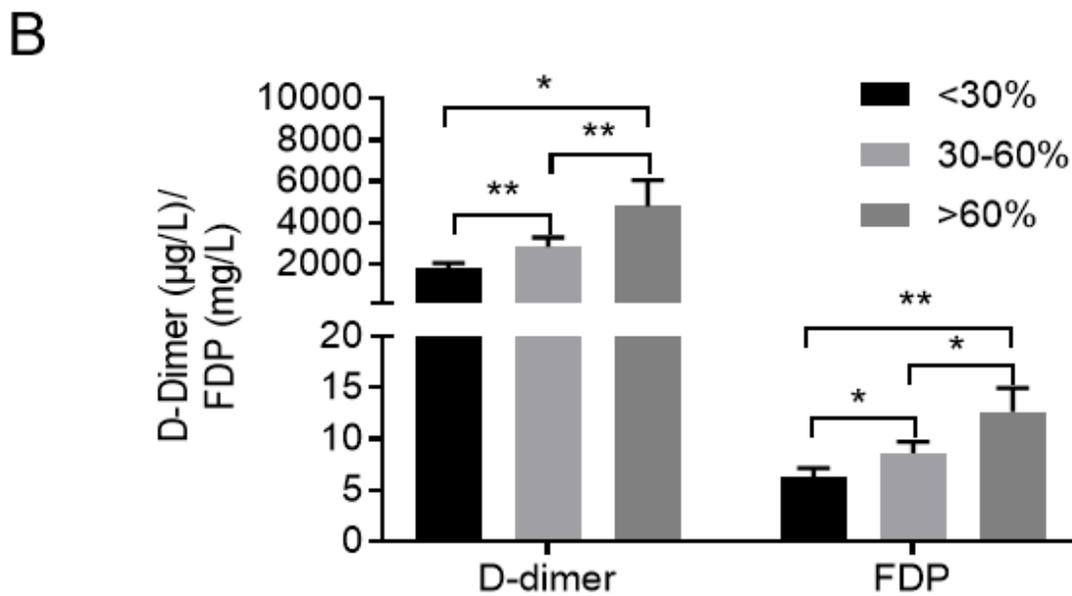
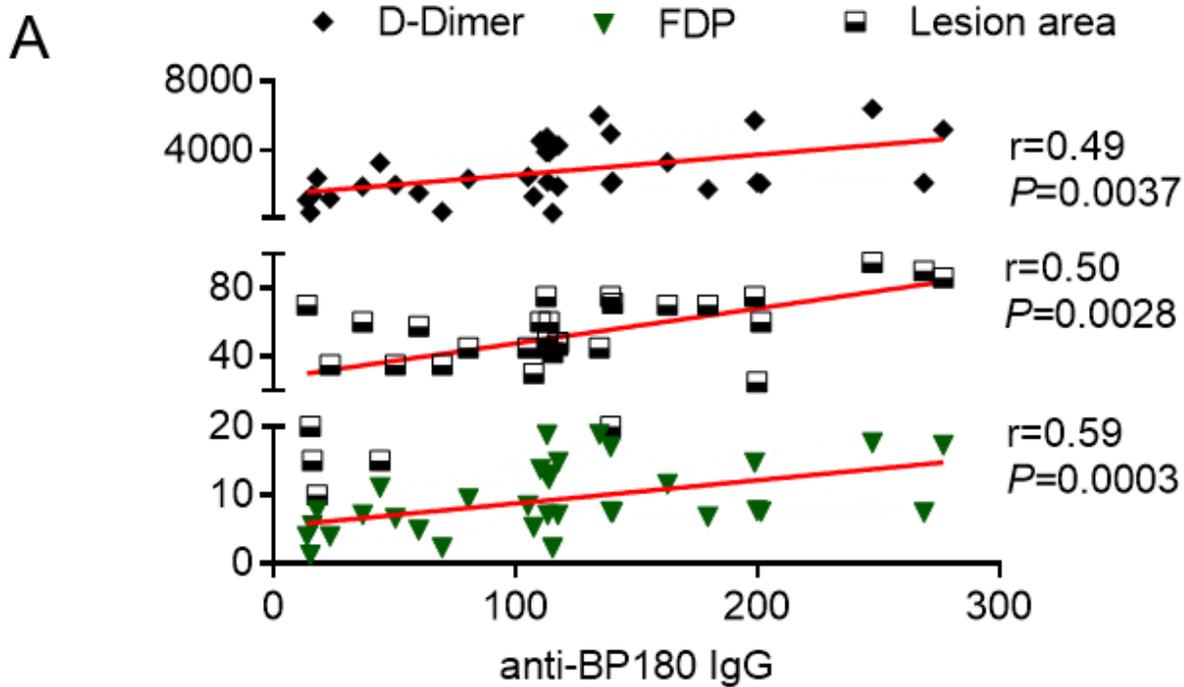


Figure 2

The correlation among D-Dimer, FDP, BP lesion area, and anti-BP180 IgG. A. Relation of anti-BP180 IgG titres and levels of anti-BP180 IgG, D-dimer, FDP and lesion area. The red line represents the trend line. B. The relation between the lesion area and levels of D-dimer and FDP. FDP, fibrinogen/fibrin degradation products; BPDAI, bullous pemphigoid disease area index.

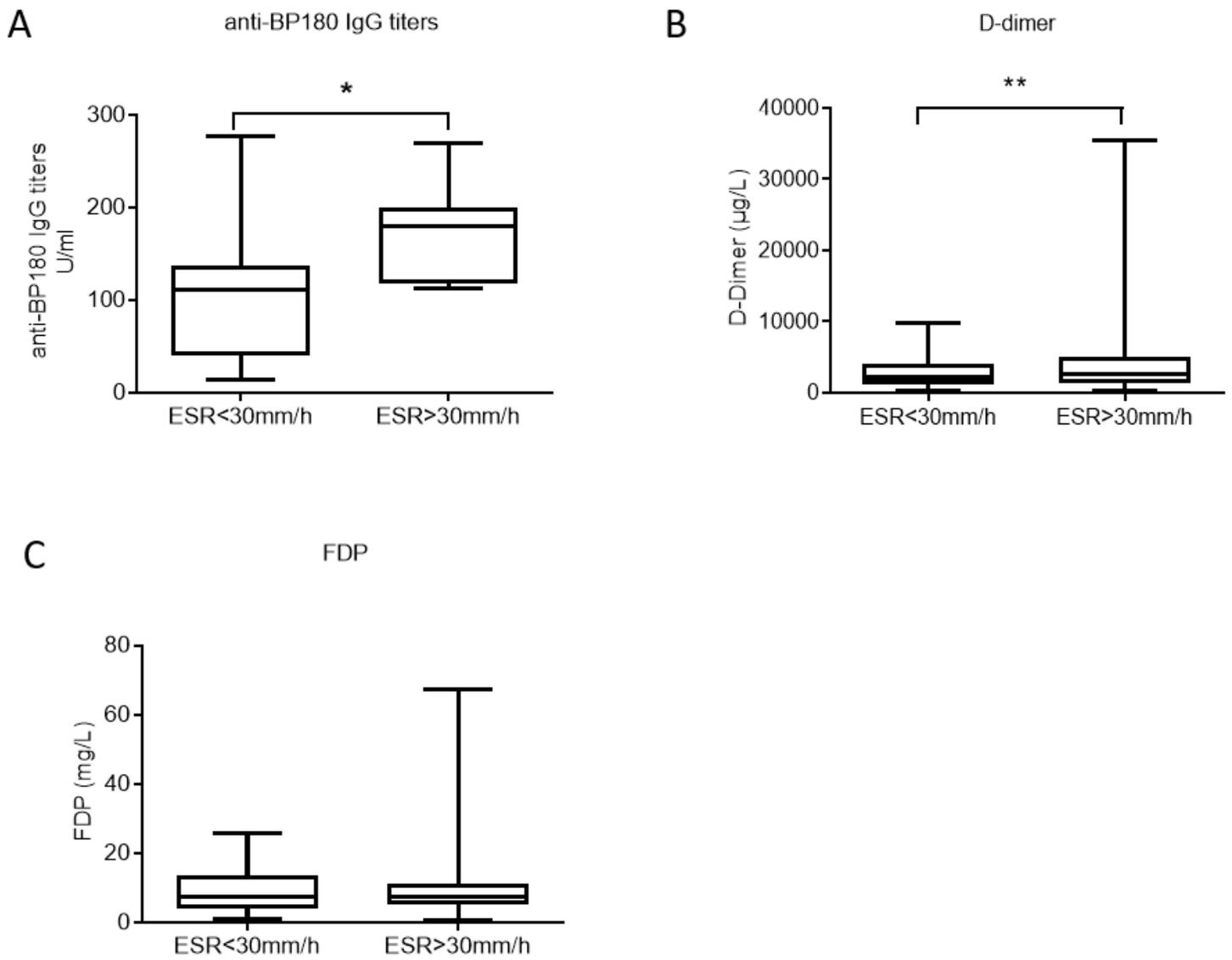


Figure 3

High D-dimer and anti-BP180 IgG levels are associated with an increased systemic inflammatory status. A. The level of anti-BP180 IgG titers, measured by ELISA, in patients with an ESR >30 mm/h (n=8), compared with patients with an ESR <30 mm/h (n=25). B. The level of D-dimer in patients with an ESR >30 mm/h (n=8), compared with patients with an ESR <30 mm/h (n=25); C. The level of FDP in patients with an ESR >30 mm/h, compared with patients with an ESR <30 mm/h. ESR, erythrocyte sedimentation rate; FDP, fibrinogen/fibrin degradation products.

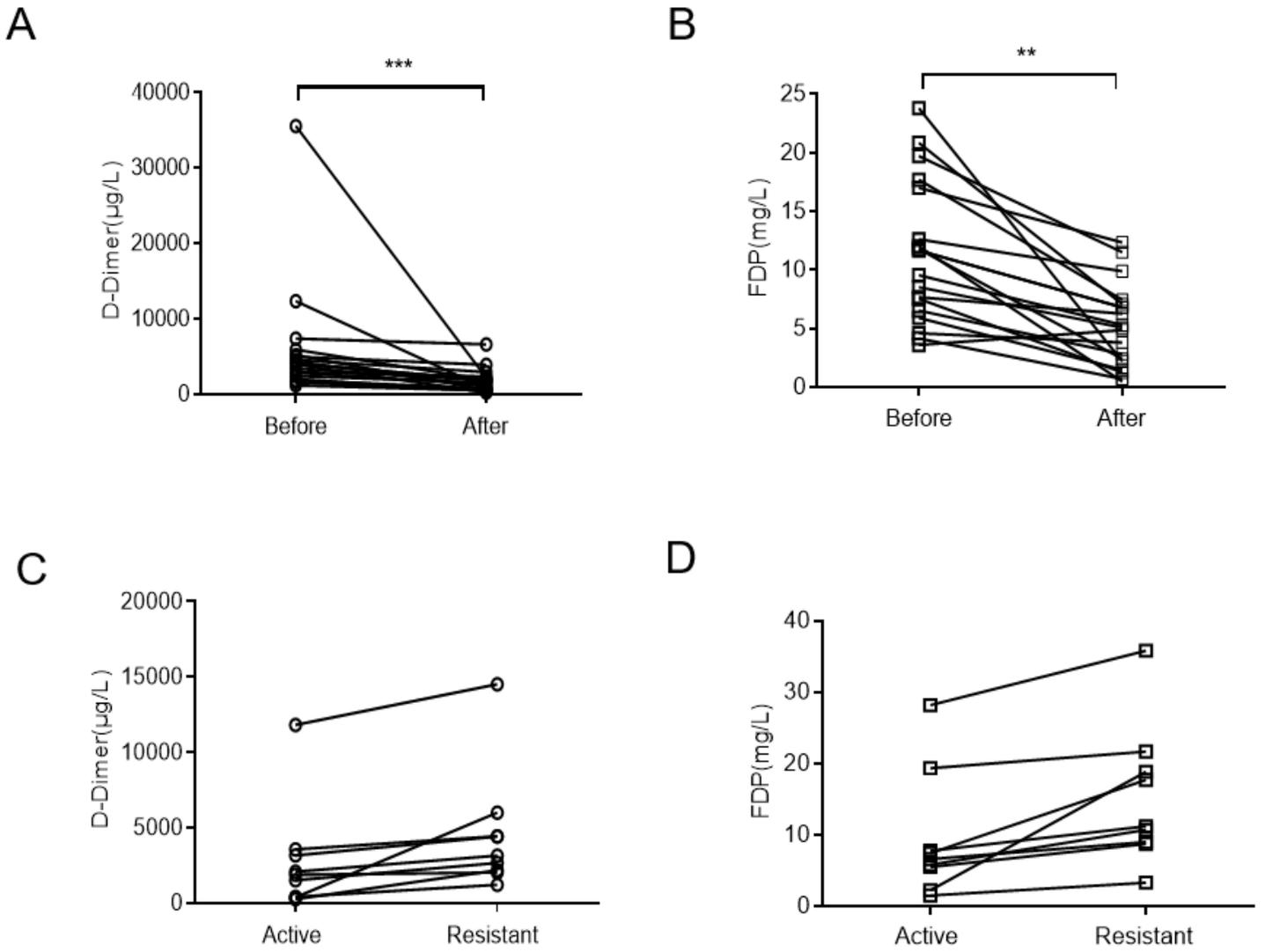


Figure 4

The change in the D-dimer and FDP in BP patients with treatment-effective and -resistant BP. Plasma D-dimer (A) and FDP (B) levels in 19 BP patients with treatment-effective BP was evaluated before and after immunosuppressive therapy, leading to complete remission. Marked reductions in both were observed upon remission. Plasma D-dimer (C) and FDP (D) levels in ten patients with treatment-resistant BP evaluated before and after the immunosuppressive therapy, which led to complete remission. A slight elevation in both was observed without relief. BP, bullous pemphigoid; FDP, fibrinogen/fibrin degradation products.

Supplementary Files

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- [figs2.png](#)

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