

Activation of coagulation, anti-coagulation, fibrinolysis and the complement system in patients with urticaria

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Summary

Background: Recently released studies indicate that activation of blood coagulation may be involved in causing urticaria.

Objective: To evaluate whether or not anticoagulation, fibrinolysis and the complement system are also involved in the pathogenesis of urticaria.

Methods: Coagulant factors, anticoagulant factors, fibrinolytic markers and complement components were analysed in patients with acute urticaria (AU) and chronic urticaria (CU).

Results: Plasma levels of activated factor VII (FVIIa) were higher in AU patients ($P < 0.01$) but not significantly different in CU patients ($P > 0.05$), while levels of the thrombin-antithrombin complex (TAT) and prothrombin fragment 1+2 (F1+2) were significantly higher in CU patients ($P < 0.01$). Levels of factor IX (FIX) and tissue factor (TF) were lower in CU patients ($P < 0.01$). Plasma levels of tissue factor pathway inhibitor/activated factor X (TFPI/Xa) were higher in CU patients ($P < 0.01$) but not significantly different in AU patients ($P > 0.05$), whereas levels of thrombomodulin (TM) were lower in CU patients ($P < 0.01$). Plasma levels of D-dimer in AU and CU patients and levels of high molecular weight kininogen (HMWK) in CU patients were increased significantly ($P < 0.01$),

while levels of tissue-type plasminogen activator (t-PA) were decreased ($P < 0.01$). Plasma concentrations of C5a in CU patients were superior to those in healthy controls ($P < 0.01$). Serum levels of C4 also increased ($P < 0.01$).

Conclusion: The activation of coagulation, anti-coagulation, fibrinolysis and the complement system may be involved in the pathogenesis of urticaria. It also indicates that coagulation conditions in CU patients can recover after antihistamine treatment, but do not immediately return to normal levels directly after administration. (*Asian Pac J Allergy Immunol* 2012;31:43-50)

Key words: urticaria, coagulation, anticoagulation, fibrinolysis, complement

Introduction

Urticaria, characterised as a recurrence of wheals, is a common condition with a significant to severe negative impact on its host's quality of life. It is estimated that 15–20% of the population suffer at least one episode of urticaria during their lifetime.¹ Urticaria can occur acutely or evolve in a chronic mode² and its treatment often stumps the dermatologist. In the last few decades, it has been generally accepted that histamine released from basophils and mast cells is at the core of the pathogenesis of urticaria. Previous studies have demonstrated an autoimmune pathogenesis mediated by active autoantibodies to the high-affinity IgE receptor (FcεRI) or to IgE,³ which are able to induce the activation of basophils and mast cells. However, some aspects of this pathogenic mechanism remain unclear. Recent studies have found that activation of the coagulation cascade resulting in thrombin production is associated with urticaria.^{4,5} This finding has particularly attracted researchers' attention as an opportunity to further our understanding of the pathogenesis of urticaria. Anticoagulant treatment is a new treatment for urticaria⁶⁻⁸ and there is little literature about the relationship of coagulation, anticoagulation and urticaria, which calls for further investigation. In

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this study, we analyse whether blood coagulation, anti-coagulation, fibrinolysis and complement are all involved in urticaria. Firstly, we measured activated factor VII (FVIIa), which is very important in initiating blood coagulation, to evaluate whether the tissue factor pathway is active. The tissue factor pathway inhibitor/activated factor X (TFPI/Xa) was also measured as a marker of activation of the extrinsic anti-coagulation system. Secondly, we measured D-dimer as the marker of fibrin generation and subsequent fibrinolysis and then measured the thrombin-antithrombin complex (TAT) and factor IX (FIX) before and after antihistamine administration. This was to see whether antihistamine drugs act in the coagulating condition in CU patients. Finally, we measured prothrombin fragments 1+2 (F1+2), tissue factor (TF), thrombomodulin (TM), high molecular weight kininogen (HMWK) and tissue-type plasminogen activator (t-PA) to further investigate the coagulation, anti-coagulation and fibrinolysis condition in urticaria. Complement system components such as C5a, C3 and C4 were also measured.

Methods

Subjects

40 patients (AU, 20, male/female: 10/10, mean age: 33 years, age range: 12-63years; CU, 20, male/female: 11/9, mean age: 38 years, age range: 17-69 years) and 20 controls (male/female: 12/8, mean age: 38 years, age range: 18-63years) were studied in July-December 2009. 70 CU patients (patients before treatment, 40, male/female: 20/20, mean age: 38 years, age range: 17-78years; patients after treatment, 30, male/female: 14/16, mean age: 42 years, age range: 21-78years) and 30 controls (male/female, 16/14, mean age, 39 years, age range, 18-60years) were studied from July 2009 to July 2010; 40 CU patients (male/female: 15/25, mean age: 39 years, age range: 16-58 years) and 30 controls (male/female: 16/14, mean age: 39 years, age range: 18-60 years) were studied from September 2010 to December 2010.

Patients were all from the 4th Zhongshan Road Clinic of Guangzhou Institute of Dermatology and Venereology (Guangzhou, China). There were no statistically significant differences in sex and age among the enrolled groups. AU was diagnosed based on the sudden appearance of wheals lasting no longer than six weeks, while CU was diagnosed based on the appearance of continuous or recurrent

wheals for more than 6 weeks. We excluded patients with lesions that were suspicious for urticarial vasculitis, patients with focal infection, physical urticaria, food allergy, or those who were taking drugs that could exacerbate CU. Patients who took immunomodulating drugs, anti-haemorrhagic drugs or anticoagulant drugs within 30 days, or patients who took antihistamine drugs within 7 days were also excluded. The scoring system was based on the assessment of key urticaria symptoms (wheals and pruritus, wheals assessment: 0 = none; 1 = mild (<20 wheals/24 h), 2 = moderate (21–50 wheals/24 h), 3 = intense (>50 wheals/24 h or large confluent areas of wheals); pruritus assessment: 0 = none; 1 = mild, 2 = moderate, 3 = intense). Patients documented 24-h self-evaluation scores.³ This study was ethically approved by a local ethical committee, in compliance with the Declaration of Helsinki. All subjects gave their informed consent before participation.

Instruments and reagents

FVIIa, TFPI/Xa, D-dimer and TM examination reagent boxes were all from American Diagnostica Inc, while TAT, FIX, TF, HMWK, t-PA were bought from American Assaypro. F1+2 and C5a were bought from Siemens and Becton, Dickinson and Company, respectively. ALISEI QUALITY SYSTEM Automated ELISA Processor was produced by SEAC, Italy.

Experimental methods

Serum levels of C3 and C4 were assayed by nephelometry immediately after samples were taken. Plasma levels of FVIIa, FIX, TF, F1+2, TAT, TFPI/Xa, TM, D-dimer, HMWK, t-PA and C5a using enzyme-linked immunosorbent assay (ELISA). Sodium citrate-anticoagulated plasma from patients with urticaria and from control subjects was stored in plastic cones at -80°C until *in vitro* assays were performed. Specimen processing was executed according to the reagent instruction booklet.

Statistical analysis

The results of normally distributed data were expressed as mean \pm standard deviation. ANOVA followed by post-hoc Dunnett's T3 was applied to compare the data among the three study groups and Kruskal-Wallis *H* Test was used when variances were unequal. An independent sample Student's *t*-test was applied to compare the data between the two study groups. The correlations were assessed

with Spearman correlation coefficients. $P < 0.05$ was taken as significant.

Results

Coagulant factors

Compared with healthy controls, plasma levels of FVIIa were higher in AU patients ($P < 0.01$) but not significantly different in CU patients ($P > 0.05$). Plasma levels of TAT and F1+2 were significantly higher ($P < 0.01$) but those of FIX and TF were lower in CU patients ($P < 0.01$). Plasma levels of TAT and FIX in CU patients tended to recover when the symptoms were controlled by antihistamine treatment, but reached no statistical difference ($P > 0.05$) (Figure 1, Figure 2 and Figure 3).

Anticoagulant factors

Compared with healthy controls, plasma levels of TFPI /Xa were higher in CU patients ($P < 0.01$), but not significantly different in AU patients ($P > 0.05$). Plasma levels of TM were lower in CU patients than those in controls ($P < 0.01$) (Figure 1, Figure 3).

Fibrinolytic markers

Compared with healthy controls, plasma levels of D-dimer increased significantly ($P < 0.01$) in AU and CU patients, and those of HMWK in CU patients also increased, whereas those of t-PA decreased ($P < 0.01$) (Figure 1, Figure 3).

Complement

Plasma concentrations of C5a and serum levels of C4 in CU patients were superior to those in healthy controls ($P < 0.01$). Serum levels of C3 were not significantly different from healthy controls ($P > 0.05$) (Figure 3, Figure 4).

Correlation analysis

There were correlations between plasma levels of F1+2, TAT, D-dimer, HMWK, t-PA and symptom scores in CU patients ($r = 0.81, P < 0.01$; $r = 0.55, P < 0.01$; $r = 0.73, P < 0.01$; $r = 0.39, P < 0.05$; $r = 0.35, P < 0.05$; respectively). Neither the parameters were correlated with the course (Table 1).

Discussion

Coagulation and urticaria

F1+2, indicators of the activation of blood coagulation, are markers of thrombin generation during blood coagulation – the measurement of F1+2 will sensitively reflect the coagulation condition.⁹ TAT is the complex of thrombin and antithrombin which is the product of the interaction between coagulation and anti-coagulation. It is also

regarded as a marker of coagulation activation and the generation of thrombin. We found that plasma levels of F1+2 and TAT in CU patients significantly exceeded that in healthy controls, paralleling other studies.^{4,5,10,11} This also suggested the presence of coagulation activation and the generation of thrombin in CU patients and indicated that thrombin may play an important role in the pathogenesis of urticaria. Thrombin, a type of serine protease, can stop bleeding, repair impaired vasculum, rebuild vasculum and repair tissue by activating protease activity receptor. Moreover, studies have shown that thrombin participates in mast cell degranulation by activating protein kinase receptors on the mast cell surface.¹² Solute permeability may result in cutis and mucosal oedema.¹³

Factor VII, the first factor of the extrinsic coagulation pathway, forms a complex with the membrane-bound tissue factor in the presence of Ca^{++} that activates primary factor X. Once thrombin is generated, it acts on fibrinogen, which is converted into fibrin that is then stabilised by factor XIIIa and finally degraded by plasmin. In this study, we found that FVIIa plasma levels were higher in AU patients, but not significantly different in CU patients; the research of Asero⁵ found that it was also elevated. The reason for this may be that the level of initiator increases once the coagulation cascade is activated. However, it may decrease gradually and, as the disease progresses through different stages, may present at different levels.

FIX, the biggest factor of the vitamin K-dependent protein family, is one of the crucial coagulation factors in the coagulation cascade. It is thought that in the traditional clotting mechanism, FIX mainly participates in either the intrinsic or the extrinsic activation pathway of coagulation. However, in this new model of coagulation, FIX plays roles in both the intrinsic and the extrinsic activation pathway. In our study, plasma levels of FIX were significantly lower than those in healthy controls; this suggests that activation of the coagulation system and the presence of FIX consumption occurs in CU patients. In the clinic, we found vitamin K to be efficient in the treatment of urticaria in some patients, which may offer some confirmatory evidence, but this requires further study.

Tissue factor, also called factor III, is the initiator in the extrinsic pathway of coagulation. The complex of TF with factor VIIa catalyses the

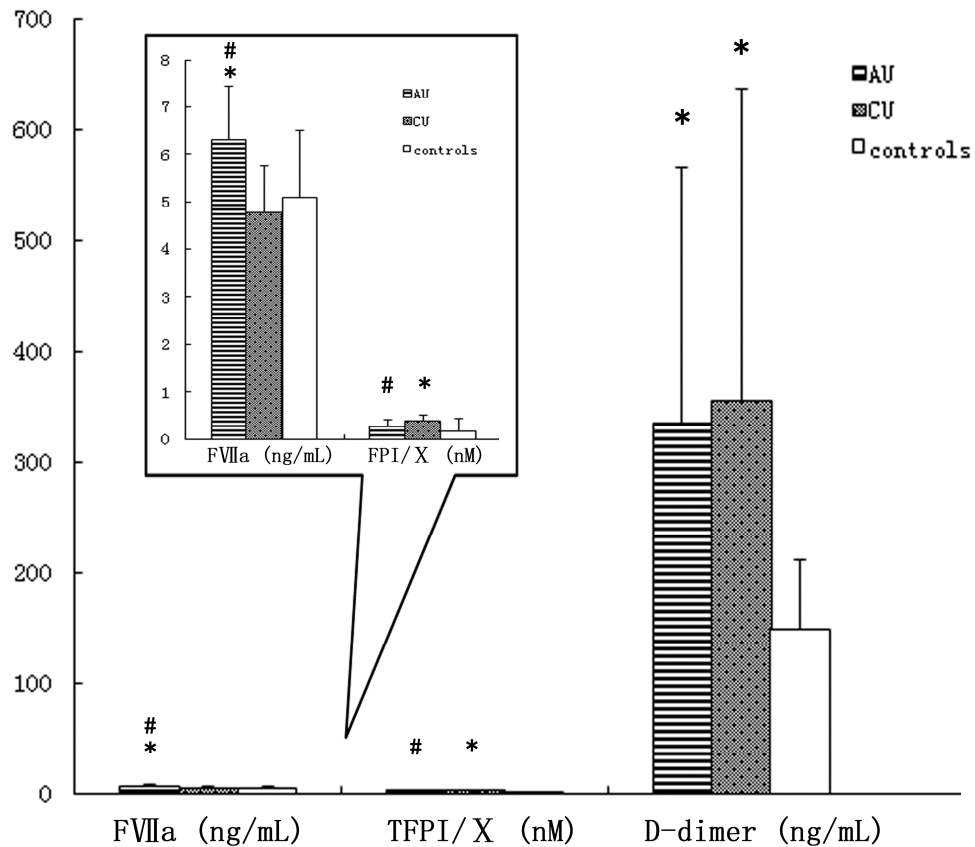


Figure 1. Results of plasma levels of FVIIa,TFPI/Xa and D-dimer in AU and CU patients. * Contrast to controls, $P < 0.01$. # AU vs. CU: $P < 0.01$.

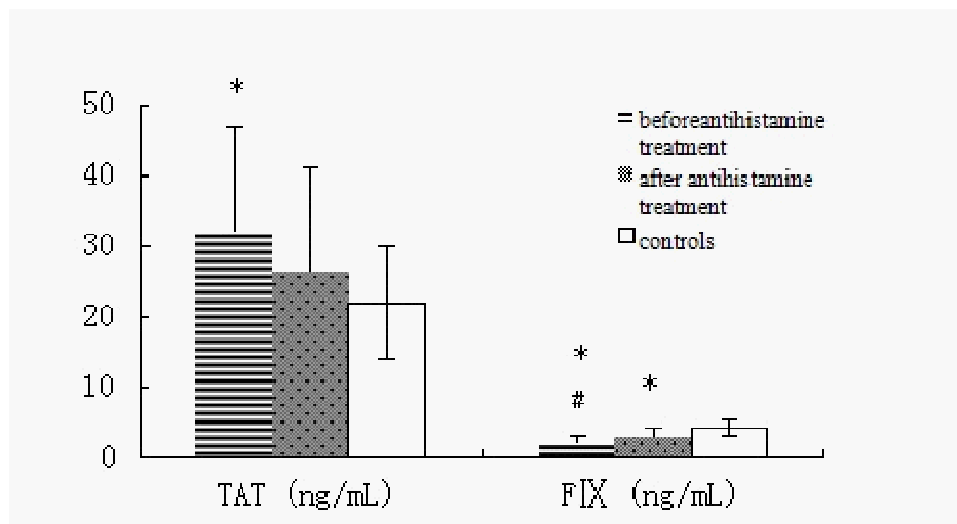


Figure 2. Results of plasma levels of TAT and FIX in CU patients. * Contrast to controls: $P < 0.01$. # CU before treatment vs. CU after treatment: $P < 0.01$.

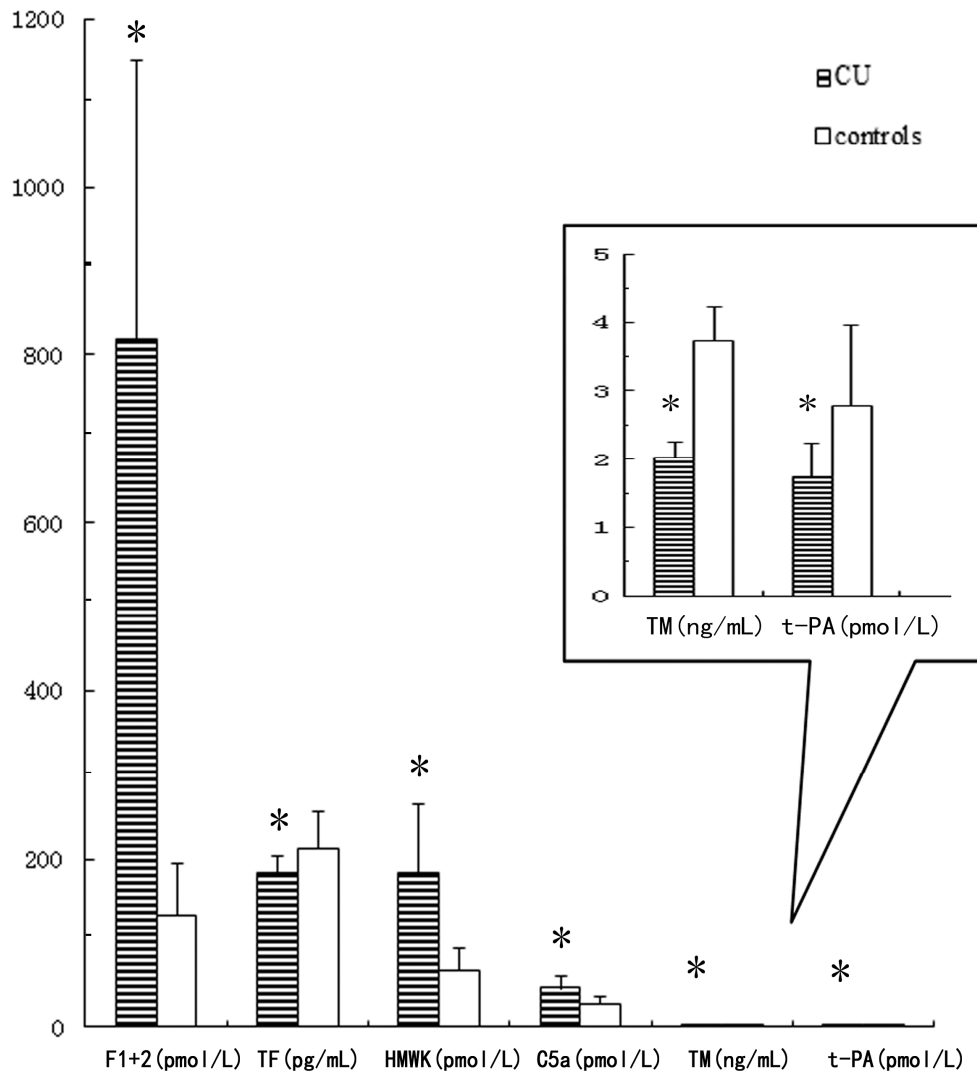


Figure 3. Plasma levels of F1+2, TF, HMWK, C5a, TM and t-PA in CU patients and controls. * $P < 0.01$.

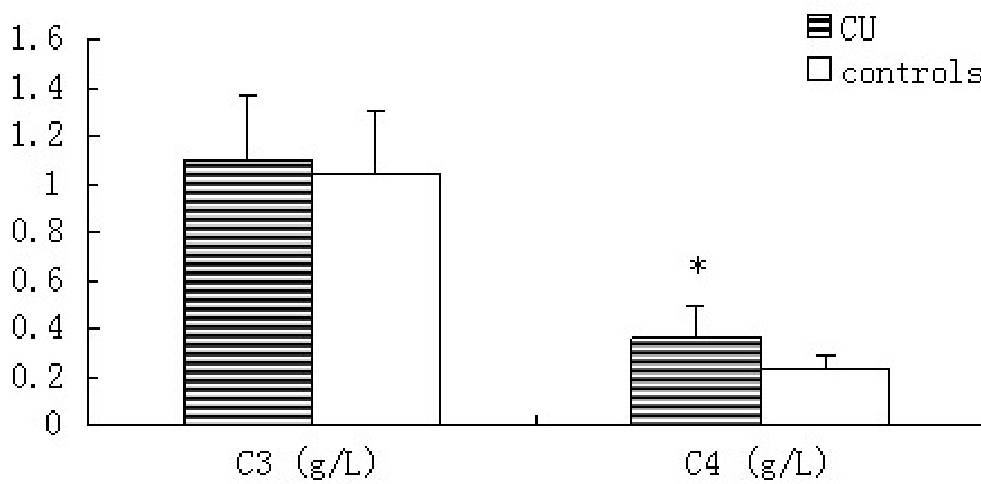


Figure 4. Results of serum levels of C3 and C4 in CU patients. * $P < 0.01$.

conversion of the inactive protease factor X into the active protease factor Xa, which then activates the extrinsic coagulation pathway. Twenty patients with severe CU were studied in another research. In that study, skin biopsy specimens, taken from wheals, were evaluated by immunohistochemical methods using an anti-TF monoclonal antibody. All specimens from patients with CU clearly showed that TF expression was absent in all normal control specimens. In addition, the double-staining experiments for TF and eosinophil cationic protein clearly showed that the TF-positive cells were eosinophils, suggesting that eosinophils expressing TF are the cell type triggering the activation of the TF pathway of coagulation in patients with CU.¹⁴ Asero and their colleagues also found the activity of TF in the lesions from CU patients in their study.⁵ Moreover, Fujii demonstrated that abundant fibrinogen deposited in the dermis of biopsy specimens from urticaria and considered that once the plasma coagulation factors extravasate into the dermis, the coagulation/fibrinolysis cascade is instantly activated.¹¹ We found out that lower concentrations of TF in CU patients may be the result of plasma TF extravasating into the dermis, and then activating the coagulation/fibrinolysis cascade.

Anti-coagulation and urticaria

Tissue factor pathway inhibitor (TFPI) is a multivalent Kunitz-type protease inhibitor that primarily inhibits the extrinsic pathway of blood coagulation. An increased level of TFPI suggests that the extrinsic pathway of blood anti-coagulation may be activated. Conversely, TM plays an important role in preventing thrombosis *in vivo* as a component in the intrinsic anti-coagulation system. The significantly lower concentration also suggests activation of the intrinsic pathway of blood anti-coagulation.

Fibrinolysis and urticaria

D-dimer is a marker of the degradation of cross-linked fibrin, and is also a well-known marker for the activation of coagulation and fibrinolysis. We found D-dimer plasma levels to be significantly elevated in patients with AU and CU, consistent with the research of others,^{5,10,11,15} which suggests that the coagulation cascade was activated in urticaria, to the degradation of cross-linked fibrin. In the research of Kasperska-Zajac,¹⁶ they found that circulating molecules of urokinase-type plasminogen activator (uPA), urokinase-type plasminogen activator soluble receptor (suPAR) and plasminogen activator inhibitor (PAI-1) were not markedly different in 16 CU patients with positive ASST as compared to 28 CU patients with negative ASST, as well as healthy subjects. They concluded that systemic fibrinolysis might not be involved in chronic urticaria. However, elevated D-dimer plasma levels in patients with urticaria suggest activation of fibrinolysis. A limited number of patients may be the reason for this, as in our study we found significantly increased HMWK and decreased t-PA concentrations in CU patients – which also suggests the activation of fibrinolysis.

Complement system and urticaria

We found that plasma levels of C5a and serum levels of C4 increased in CU patients suggesting activation of the complement system. This demonstrates a new complement activation pathway where C5a generated in the absence of C3 by thrombin takes the place of C5 convertase in the classical pathway.¹⁷ Pathogenic IgG cross-links the IgE receptor directly to cause histamine release, and activation is augmented by complement. C5a is the complement agonist that is responsible for the augmented histamine release.¹⁸ For the past 20 years, it has been known that histamine release is initiated by cross-linking of α subunit of Fc ϵ RI by

Table 1. Correlation with FVIIa, TFPI /Xa, D-dimer, TAT, F1+2, HMWK and t-PA in chronic urticaria patients

Subject	FVIIa	TFPI /Xa	D-dimer	TAT	F1+2	HMWK	t-PA
score							
r	-0.094	0.17	0.73	0.55	0.81	-0.39	0.35
P	0.64	0.39	<0.01	<0.01	<0.01	0.01	0.03
course							
r	0.15	-0.33	0.03	0.03	0.136	-0.09	0.1
P	0.46	0.09	0.9	0.89	0.57	0.72	0.65

means of IgG antibody, which is the major immunologic mechanism in chronic urticaria. What is the relationship between the present finding and the traditional immunologic mechanism? It has been proposed that CU is a disease at the crossroads between autoimmunity and coagulation.¹⁹ We have hypothesised that generation of thrombin, complement and pathogenic IgG may play significant roles in the pathogenesis of CU.

Correlation analysis with symptom score and course

There are correlations between plasma levels of F1+2, TAT, D-dimer, HMWK and t-PA and symptom scores in CU patients in this study. Combined with other studies,^{10,13,15,25} we consider that F1+2, TAT, and D-dimer levels are significantly related to disease severity. They may be the excellent markers which can reflect the coagulation/fibrinolysis condition in CU patients, but whether they can act as the parameters for evaluation of disease severity and therapeutic effect still needs further investigation.

Antihistamine drugs and coagulation condition

When the symptoms were under control after antihistamine treatment, plasma levels of FIX and TAT tend to recover but they were still lower than healthy controls. It is possible that the course of antihistamine treatment is too short in our study to make the coagulation imbalance recover. We have discussed the fact that thrombin participates in mast cell degranulation by activating the protein kinase receptor on the mast cell surface.¹² Solute permeability may result in cutis and mucosal oedema^[13]. However, this effect can be blocked by antihistamine drug administration or mast cell granule attenuation. In contrast, mast cell recruitment and activation may result in local thrombolysis and the prevention of coagulation.²⁰

In conclusion, this study shows that the activation of coagulation, anti-coagulation, fibrinolysis and the complement system may all be involved in the development and progression of urticaria. However, not all of the related proteins maintain similar levels as the disease progresses. Plasma levels of some coagulant and anticoagulant factors may change through the different stages of urticaria. F1+2, D-dimer and TAT may not only be stable parameters that reflect coagulation and fibrinolysis, but may also be associated with symptom scores in urticaria. We consider that activation of the coagulation cascade results in

generation of thrombin, subsequently activating anti-coagulation and fibrinolysis; thrombin and other proteins, in the course of this activation, participate in mast cell degranulation and result in urticaria. Furthermore, coagulation conditions in patients with CU can recover after antihistamine treatment but do not completely return to normal levels immediately after administration. How the coagulation, anti-coagulation, fibrinolysis and complement systems act in CU pathogenesis, and the relationship between the present finding and the traditional immunologic mechanism still needs further investigation.

Conflicts of interest

All authors have no conflicts of interest in this study.

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