

Evaluation of autologous plasma skin test in patients with chronic idiopathic urticaria

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Summary

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Background The pathophysiological mechanism of chronic urticaria is still poorly understood and its aetiology is considered to have an autoreactive basis. Autologous serum skin tests (ASSTs) and autologous plasma skin tests (APSTs) comprise the simplest ways for diagnosing autoreactive urticaria (with autoantibodies, histamine-releasing factor and coagulation factors, especially thrombin) in a clinical setting. However, there are still some questions about the specificity of these tests.

Objectives To evaluate the role of autoreactivity in the pathogenesis of chronic urticaria by means of measuring plasma prothrombin fragments 1 + 2, which are used as markers of thrombin, and to compare the APST with the ASST.

Methods Forty-two patients (19 men and 23 women; mean age 35.7 years, range 28–76) and 35 healthy volunteers (19 men and 16 women; mean age 30.3 years, range 20–80) were included in the study. APST, negative (ASST, sodium citrate, saline) and positive (histamine) control tests were performed in the patient and control groups. The levels of plasma prothrombin fragments 1 + 2 were also assessed. **Results** When the APST was evaluated without negative controls, it was positive in 67% of patients. However, the APST was positive in 0% when it was evaluated with negative controls. Levels of prothrombin fragments 1 + 2 were found to be elevated in patients with chronic idiopathic urticaria.

Conclusions We suggest that as negative control tests were not performed along with the APST in previous studies, the APST showed a high rate of positivity. Thus, the use of APST for evaluating autoreactivity in clinical practice is not superior to the use of ASST and further studies should be conducted.

Chronic urticaria (CU) is a skin disorder which is characterized by the recurrent eruption of short-lived weals accompanied by redness and itching for a minimum of 6 weeks.¹ Weals may be associated with angio-oedema. The pathogenesis of the disease is still poorly understood. Several studies suggest that the aetiology of CU has an autoimmune basis. The autologous serum skin test (ASST), based on the intradermal injection of autologous serum, is a simple screening diagnostic procedure used in chronic autoimmune urticaria (CAU). A positive ASST result suggests that there are functional autoantibodies (anti-FcεRI and/or anti-IgE) or histamine-releasing factors (HRFs) in the serum. Several studies showed ASST positivity in almost half of the patients with chronic idiopathic urticaria (CIU). However, the actual pathophysiological mechanism is still unknown.^{2–9}

Recent studies demonstrated activation of the coagulation cascade in CU.^{10–12} Thrombin and prothrombin fragments 1 + 2 (F₁₊₂) are developed from prothrombin in the extrinsic

coagulation cascade, activated by the tissue factor released from inflammatory cells.⁵ Thrombin is a serine protease acting in both the extrinsic and the intrinsic coagulation systems.^{13,14} Thrombin is the final enzyme of the coagulation system, which is capable of increasing vascular permeability, producing C5a (anaphylatoxin), and activating and degranulating mast cells. Patients with CU often present with elevation of coagulation and fibrinolysis markers, such as F₁₊₂ and D-dimer, which relate to the disease severity.^{10,15} It was also reported that some patients with CU responded to anticoagulant therapies such as heparin and warfarin sodium.^{16,17} In this regard, the coagulation cascade plays an important role in the pathogenesis of CIU.

The aim of this study was to evaluate the role of autoreactivity in the pathogenesis of CU, by means of measuring plasma F₁₊₂ which are used as markers of thrombin, and to compare the autologous plasma skin test (APST) with the ASST as *in vivo* screening tests in clinical practice.

Materials and methods

Patients

Forty-two patients and 35 healthy volunteers were included in the study. Patients comprised 19 men and 23 women, age range 28–76 years, mean 35.7. Controls comprised 19 men and 16 women, age range 20–80 years, mean 30.3. The control group had no history of atopy (atopic respiratory disease, atopic dermatitis or food allergy), urticaria or any drug allergy and/or intolerance. All patients had stopped any medication (antihistamines, corticosteroids etc.) at least 7 days before intradermal tests were performed. The exclusion criteria for patients with CIU were physical urticaria, pregnancy and urticaria lasting < 6 weeks. CU was diagnosed based on the appearance of continuous or recurrent hives with/without angio-oedema that lasted longer than 6 weeks. All participants underwent the following tests: blood cell count and differential, erythrocyte sedimentation rate, nasal smear, serum total IgE concentration, free triiodothyronine, free thyroxine, thyroid-stimulating hormone, antithyroid peroxidase, antithyroglobulin, hepatitis B surface antigen, anti-Hbs, antihepatitis C virus, human immunodeficiency virus, C-reactive protein, hepatic and renal function tests, urinalysis, antinuclear antibody, rheumatoid factor, C3, C4, parasitological examination and occult blood in stool, Waters view X-ray, and chest X-ray. APST, ASST, sodium citrate, saline and histamine skin tests (SC, S and H, respectively) were also performed in the participants. Disease activity was evaluated based on the number of weals present at the time when blood samples were collected, and described as follows:^{10,11} grade 1 (mild), 1–10 small (< 3 cm diameter) weals; grade 2 (moderate), 10–50 small or 1–10 large (> 3 cm diameter) weals; grade 3 (severe), > 50 small or > 10 large weals; grade 4 (very severe), virtually covered with weals.

The study protocol complied with the ethical guidelines of the Declaration of Helsinki of the World Medical Association and was approved by the Hospital's Medical Ethics Committee.

Skin tests

APST, ASST, SC, S and H tests were performed in both the patient and the control groups. Typically, both serum and plasma contain autoantibodies. However, plasma (including the added amount of sodium citrate) also contains certain coagulation factors and proteins (factor I, II, IV, VII, VIII, IX, X, XII, XIII, protein C, protein S, antithrombin III, fibronectin) which play a role in the coagulation cascade. On the other hand, serum contains neither coagulation factors nor proteins (Table 1).¹⁴

Autologous plasma skin test

In order to prepare plasma and measure F_{1+2} levels, 4 mL venous blood samples of both groups were collected in sterile vacutainers (PTZ tubes, BD Vacutainer Systems®; BD, Franklin

Table 1 Differences between serum and plasma

	Serum	Plasma
Autoantibodies	+	+
Histamine-releasing factor	+	+
Coagulation factors and proteins ^a	–	+
Sodium citrate	–	+

^aFactor I, II (IIa = thrombin), IV, VII, VIII, IX, X, XII, XIII, protein C, protein S, antithrombin III, fibronectin.

Lakes, NJ, U.S.A.) containing 0.5 mL of 0.105 mol L⁻¹ sodium citrate. Blood samples containing 1/9 citrate (0.5 mL sodium citrate + 4 mL venous blood) were kept at room temperature for 15 min, and then centrifuged for 3 min at 1250 g to obtain plasma.

Sodium citrate skin test

The SC test is the negative control of the APST. In order to prepare an equal concentration of SC test solution (1/9: 0.5 mL sodium citrate + 4 mL saline) as in the APST solution (1/9: 0.5 mL sodium citrate + 4 mL venous blood), 4 mL sterile 0.9% saline was added into another sterile vacutainer (PTZ tubes, BD Vacutainer Systems®) containing 0.5 mL of 0.105 mol L⁻¹ sodium citrate.

Autologous serum skin test

Venous blood samples were drawn into sterile tubes without clotting (Vacutest®; BD). The blood samples were allowed to clot at room temperature for 30 min, and centrifuged at 500 g for 15 min to separate the sera.⁶ Then 0.05 mL plasma, autologous serum, and 1/9 diluted sodium citrate were injected intradermally through the volar aspects of the forearms.

Saline skin test

Sterile 0.9% saline (as negative control) was also injected intradermally through the volar aspect of the forearm.

Histamine skin test

A skin prick test with histamine (10 mg mL⁻¹) was performed as a positive control.⁶ All the above tests were evaluated by the same investigator.

Evaluation of data

The ASST was interpreted as positive if a serum-induced weal was red and had a diameter bigger than a saline-induced response by ≥ 1.5 mm at 30 min.² The APST was interpreted as positive if a plasma-induced weal-and-flare had a diameter > 3 mm and the ASST, S and SC tests were negative.^{3–10}

Measurement of prothrombin fragments 1 + 2

Sodium citrate-anticoagulated plasma samples obtained from 42 patients with CIU and from 35 healthy subjects were stored in Eppendorf tubes at -80°C and F_{1+2} were measured by means of ELISA Kit Human Prothrombin F_{1+2} (Uscn Life Science Inc., Wuhan, China).

Statistical analysis

The χ^2 test, Mann–Whitney U-test and logistic regression analysis were used with SPP software version 13.0 (SPSS Inc., Chicago, IL, U.S.A.). $P < 0.05$ was considered significant.

Results

Forty-two patients and 35 age- and gender-matched healthy subjects were included in the study. Results of the APST and its negative controls (ASST, SC and S) are presented in Table 2. Nobody reacted to intradermal S injection. However, the skin prick test with H was positive in both groups.

CAU was defined as ASST(+), S(–), H(+) and CIU as ASST(–), S(–), H(+). Twenty-six of 42 patients (62%) were ASST positive. Significantly more patients with CU showed ASST positivity [ASST(+), S(–), H(+)] than individuals in the control group ($P = 0.016$). However, no individual ASST- or APST-positive case [APST(+), ASST(–), SC(–) or APST(–), ASST(+), SC(–)] was found in the patient or control groups. In this study, neither patients with CIU nor participants in the control group were found APST positive, when ASST, SC and S tests were negative. Seven (25%) SC tests were evaluated as positive in 28 APST-positive patients. Four (27%) SC tests were evaluated as positive in 15 APST-positive healthy participants.

F_{1+2} levels in nine patients with mild and one patient with moderate urticaria (based on urticaria symptom score) were compared with the levels in 35 healthy controls. The difference was found to be statistically highly significant ($P = 0.001$) (Table 3).

Table 2 Test results in patients with chronic urticaria and healthy subjects

Patients (n = 42), n (%)	APST	ASST	SC	Control group (n = 35), n (%)
14 (33)	–	–	–	20 (57)
2 (5)	+	–	+	3 (9)
5 (12)	+	+	+	1 (3)
21 (50)	+	+	–	11 (31)

APST, autologous plasma skin test; ASST, autologous serum skin test; SC, sodium citrate skin test. Histamine skin test was evaluated as positive and saline skin test was evaluated as negative in both groups.

Table 3 Plasma levels of prothrombin fragments 1 + 2 (F_{1+2}) in patient and control groups

	F_{1+2} (mean $\pm$ SD)
CU patients with lesions (n = 10)	1.9258 ± 0.6148
CU patients without lesions (n = 32)	0.4942 ± 0.4113
Healthy controls (n = 35)	0.8718 ± 0.7337

CU, chronic urticaria.

Discussion

Autoantibodies of IgG structure, developed against high-affinity IgE receptors on mast cells and basophils or against IgE bonded with those receptors, were found in approximately 50% of patients with CU. Such autoantibodies are functional and have the potential to induce histamine release from these cells. As a result, autoimmunity may play a significant role in the pathogenesis of CU and the ASST is considered a screening test for CAU.^{2–4,6}

In this study, the ASST was performed in both patient and control groups, and the difference between the groups was statistically significant ($P = 0.016$). In patients with CU, the ASST was found positive at a rate of 62%, consistent with other studies. The ASST shows large variations. Studies in patients with CU indicated an ASST positivity of 30–60%.^{4,6} The frequency of positive ASST responses of healthy controls in published studies ranges from 0% to 52.5%.¹⁸ There are some questions regarding the sensitivity and specificity of ASST and APST. Sensitivity and specificity results of ASSTs which were evaluated by means of various criteria were compared with serum-induced histamine release assays (basophil or mast cell based) by Konstantinou *et al.*¹⁹ Sensitivity was found to be between 44.4% and 100%, and specificity between 27.2% and 86.1%.

Some *in vitro* studies showed that serum of patients induced histamine release from not only basophils, but also from mast cells in 5–10% of patients with CU.^{7,8} This substance with a non-IgG structure specific to the mast cell is referred to as HRF.²⁰

Recently, a new autoreactive mechanism of thrombin origin, developed through activation of the coagulation system, has been discussed. An activated coagulation cascade induces thrombin production. Thrombin, the final enzyme of the intrinsic and extrinsic coagulation systems, is a serine protease and is capable of increasing permeability, producing C5a (anaphylatoxin), and activating and degranulating mast cells. It is considered to be active in CU pathogenesis due to such mechanisms.^{13,21,22}

Asero *et al.*¹⁰ observed that 71 of 96 patients (53%) were ASST positive and 61 of 71 patients (86%) were APST positive in a study of 96 patients with CU. Plasma F_{1+2} levels in patients with CU were found to be significantly higher than in the control group. F_{1+2} levels of APST- and ASST-positive patients were found to be higher than those of APST- and

ASST-negative patients ($P = 0.058$), and increased in direct proportion to the severity of the disease ($r = 0.37$; $P < 0.05$). Ten patients were further tested with saline containing sodium citrate at a concentration of 0.125 mol L^{-1} following the study; however, no test was performed as a negative control of APST.

However, in another multicentre study, ASST positivity was 37.5% and APST positivity was 43% among 200 patients (146 women and 54 men) with CIU.²³ These results are in contrast to the previous report of Asero *et al.*¹⁰

It is known that both plasma and serum used in APST and ASST contain autoantibodies (developed against Fc ϵ RI and IgE) and HRFs. Plasma used in APST also contains coagulation factors and sodium citrate (Table 1). Therefore, APST positivity may originate from thrombin and/or sodium citrate as well as autoantibodies. ASST and negative controls S and SC should be all negative to consider APST positive in terms of a different autoreactivity produced by coagulation factors.

As dipotassium ethylenediamine tetraacetic acid produced nonspecific reactions and heparin blocked *in vivo* and *in vitro* degranulation of basophils and mast cells in some studies, sodium citrate was used as anticoagulant in this study.^{3,23} There were no patients or healthy volunteers whose results were ASST(-), SC(-), S(-), APST(+), H(+). All APST-positive patients were also determined as SC and/or ASST positive.

APST showed 67% positivity in the patient group when it was evaluated without negative controls. However, its positivity was 0% when APST was evaluated with negative controls. Similarly, APST showed 43% positivity in the control group when it was evaluated without negative controls; however, its positivity was also 0% when APST was evaluated with negative controls. Both serum and plasma contain autoantibodies and HRF. Thereby, APST positivity may also be expected in any ASST-positive patient as in this study.

Thrombin and F_{1+2} are products of prothrombin degradation. F_{1+2} levels are used as a marker of thrombin.^{13,24} D-dimer, F_{1+2} levels and histamine release activity (gold standard test for CAU) were assessed by Asero *et al.*¹⁰ in 2006 to evaluate the role of the coagulation system in the aetiopathogenesis of CU. The mean level of F_{1+2} in plasma was 0.80 in the control group ($n = 27$) and 3.07 in patients with CU. Consequently, Asero *et al.* determined a statistically significant increase in D-dimer and F_{1+2} levels in parallel with the severity of the disease.

F_{1+2} , FXIIa, FVIIa and D-dimer levels were measured by Asero *et al.* The mean $\pm$ SD F_{1+2} was 2.54 ± 0.26 in patients with CU vs. 0.87 ± 2.57 in the control group ($P = 0.0001$). High levels of D-dimer ($P = 0.007$) and FVIIa ($P = 0.0001$) were also found to be statistically significant.¹¹ As a result, D-dimer, F_{1+2} and FVIIa levels showed a significant increase in patients with CU with active lesions. The results paralleled disease severity in this study. Plasma levels of FXIIa were similar in all patients and healthy subjects.¹¹ These data indicate that the extrinsic coagulation system is activated in patients with CU.

In the study made by Asero *et al.*³ in 2007, when immuno-histochemical analyses of skin biopsies collected from 10 patients with CU were compared with the analyses of the control group, tissue factor expression increased in epidermal inflammatory cells of the patients. Therefore, the authors suggest that activation of the extrinsic coagulation system causes an increase in thrombin which may play an important role in the pathogenesis of CIU.

In our study, the mean $\pm$ SD plasma level of F_{1+2} in 10 patients with CU with active lesions (nine mild, one moderate urticaria) was 1.9 ± 0.6 , compared with 0.5 ± 0.4 in 32 patients with CU without active lesions and 0.9 ± 0.7 in the control group ($P = 0.001$). This suggests that thrombin (the product of the extrinsic coagulation cascade) plays a role in the pathogenesis of CIU. F_{1+2} levels were in the normal range in patients with CIU without active lesions, even though they were 43% lower compared with the healthy control group. It is thought that the lower level of thrombin may be due to its consumption in the coagulation cascade.

For the first time, SC, ASST and S were all used together as negative controls of APST in all patients and controls to evaluate autoreactivity. Because previous studies did not evaluate the results based on negative controls, the positivity of APST was determined to be very high. SC, ASST and S tests should be performed as negative controls when evaluating APST. This study suggests that the high positivity of APST may result from the presence of sodium citrate. Our study seems to be a more rational approach to study the mechanisms activating the coagulation cascade to evaluate autoreactivity. We observed no statistically significant difference between APST and ASST in terms of positivity. So, we conclude that APST is not superior to ASST in determination of autoreactivity, and both can be used separately for this purpose. Further studies with negative controls are needed.

What's already known about this topic?

- Autologous serum and plasma skin tests are the simplest ways for diagnosing chronic autoimmune urticaria in the clinical setting. However, there are still some questions about the specificity of these tests.
- In a previous study, the autologous plasma skin test was found to be superior to the autologous serum skin test to assess autoreactivity in chronic idiopathic urticaria. However, that study did not include negative control tests in all of the patients.

What does this study add?

- In our study we used negative controls in all of the patients and controls, and concluded that the use of the autologous plasma skin test for evaluating autoreactivity in clinical practice is not superior to use of the autologous serum skin test for the same purpose.

References

- Kaplan AP, Greaves M. Pathogenesis of chronic urticaria. *Clin Exp Allergy* 2009; **39**:777–87.
- Sabroe RA, Grattan CE, Francis DM *et al.* The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999; **140**:446–53.
- Asero R, Riboldi P, Tedeschi A *et al.* Chronic urticaria: a disease at a crossroad between autoimmunity and coagulation. *Autoimmun Rev* 2007; **7**:71–6.
- Grattan CE. Autoimmune urticaria. *Immunol Allergy Clin North Am* 2004; **24**:163–81, v.
- Cugno M, Marzano AV, Asero R *et al.* Activation of blood coagulation in chronic urticaria: pathophysiological and clinical implications. *Intern Emerg Med* 2010; **5**:97–101.
- Fiebiger E, Maurer D, Holub H *et al.* Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J Clin Invest* 1995; **96**:2606–12.
- Niimi N, Francis DM, Kermani F *et al.* Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. *J Invest Dermatol* 1996; **106**:1001–6.
- Sabroe RA, Fiebiger E, Francis DM *et al.* Classification of anti-Fc-epsilonRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002; **110**:492–9.
- Grattan CE, Sabroe RA, Greaves MW. Chronic urticaria. *J Am Acad Dermatol* 2002; **46**:645–57.
- Asero R, Tedeschi A, Riboldi P, Cugno M. Plasma of patients with chronic urticaria shows signs of thrombin generation, and its intradermal injection causes wheal-and-flare reactions much more frequently than autologous serum. *J Allergy Clin Immunol* 2006; **117**:1113–17.
- Asero R, Tedeschi A, Coppola R *et al.* Activation of the tissue factor pathway of blood coagulation in patients with chronic urticaria. *J Allergy Clin Immunol* 2007; **119**:705–10.
- Cugno M, Marzano AV, Tedeschi A *et al.* Expression of tissue factor by eosinophils in patients with chronic urticaria. *Int Arch Allergy Immunol* 2009; **148**:170–4.
- Bauer KA, Weitz JI. Laboratory markers of coagulation and fibrinolysis. In: *Hemostasis and Thrombosis: Basic Principles and Clinical Practice* (Colman RW, Hirsh J, Marder VJ, Clowes AW, George JN, eds), 4th edn. Philadelphia, PA, U.S.A.: Lippincott Williams & Wilkins, 2001; 1113–29.
- Cohen H, Kernoff PB. ABC of transfusion. Plasma, plasma products, and indications for their use. *BMJ* 1990; **300**:803–6.
- Asero R, Tedeschi A, Riboldi P *et al.* Severe chronic urticaria is associated with elevated plasma levels of D-dimer. *Allergy* 2008; **63**:176–80.
- Chua SL, Gibbs S. Chronic urticaria responding to subcutaneous heparin sodium. *Br J Dermatol* 2005; **153**:216–17.
- Parslew R, Pryce D, Ashworth J, Friedmann PS. Warfarin treatment of chronic idiopathic urticaria and angio-oedema. *Clin Exp Allergy* 2000; **30**:1161–5.
- Taskapan O, Kutlu A, Karabudak O. Evaluation of autologous serum skin test results in patients with chronic idiopathic urticaria, allergic/non-allergic asthma or rhinitis and healthy people. *Clin Exp Dermatol* 2008; **33**:754–8.
- Konstantinou GN, Asero R, Maurer M *et al.* EAACI/GA(2)LEN task force consensus report: the autologous serum skin test in urticaria. *Allergy* 2009; **64**:1256–68.
- Claveau J, Lavoie A, Brunet C *et al.* Chronic idiopathic urticaria: possible contribution of histamine-releasing factor to pathogenesis. *J Allergy Clin Immunol* 1993; **92**:132–7.
- Cirino G, Cicala C, Bucci MR *et al.* Thrombin functions as an inflammatory mediator through activation of its receptor. *J Exp Med* 1996; **183**:821–7.
- Asero R, Tedeschi A, Lorini M *et al.* Chronic urticaria: novel clinical and serological aspects. *Clin Exp Allergy* 2001; **31**:1105–10.
- Metz M, Giménez-Arnau A, Borzova E *et al.* Frequency and clinical implications of skin autoreactivity to serum versus plasma in patients with chronic urticaria. *J Allergy Clin Immunol* 2009; **123**:705–6.
- Jackson JM. Mechanisms of prothrombin activation in hemostasis and thrombosis. In: *Hemostasis and Thrombosis: Basic Principles and Clinical Practice* (Colman RW, Marder VJ, Clowes AW, George JN, Goldhaber SZ, eds), 5th edn. Philadelphia, PA, U.S.A.: Lippincott Williams & Williams, 2006; 135–47.