

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

Riccardo Asero, MD,^a Alberto Tedeschi, MD,^b Piersandro Riboldi, MD,^{c,d} and Massimo Cugno, MD^{b,d} Paderno Dugnano (MI) and Milano, Italy

Background: Several aspects of the pathogenesis of chronic urticaria (CU) remain contradictory. Autologous serum skin tests (ASSTs) and *in vitro* histamine release assays seem to look into distinct aspects of the disease, and the specificity of ASST has been questioned.

Objective: We compared the autologous plasma skin test (APST) with ASST to detect autoreactivity in patients with CU. The clotting process was investigated as well by measuring *in vivo* thrombin generation.

Methods: A total of 96 adults with CU underwent ASST; 71 of them underwent APST with Na citrate–anticoagulated plasma. Prothrombin fragment 1+2 plasma levels were measured by a sandwich ELISA in Na citrate–anticoagulated plasmas from 28 patients and 27 controls.

Results: Fifty-one of 96 (53%) patients scored positive on ASST, whereas 61 of 71 (86%) patients scored positive on APST (21/30 [70%] ASST-negative and 40/41 [98%] ASST-positive). Plasma prothrombin fragment 1+2 was higher in patients than controls (3.06 [SD 3.36] vs 0.80 [0.34]; $P < .001$) and in ASST-positive/APST-positive than in ASST-negative/APST-positive patients (3.89 [SD 3.68] vs 1.33 [1.64]; $P = 0.058$) and was directly related to urticaria severity ($r = 0.37$; $P < .05$).

Conclusion: Most patients with CU are positive on APST–Na citrate. CU is associated with the generation of thrombin, a serine protease able to activate mast cells and to cause relevant increase in permeability of endothelium. APST and ASST only partially depend on the presence of circulating antibodies to FcεRI or to IgE.

Clinical implications: These findings provide new insights into the pathogenesis of CU and suggest new therapeutic opportunities for treating this disease. (J Allergy Clin Immunol 2006;117:1016–1022.)

Key words: Chronic urticaria, skin tests, histamine release, plasma, histamine releasing factors

Chronic urticaria (CU) is a rather common skin disorder characterized by the recurrent eruption of short-lived wheals accompanied by redness and itching for at least 6 weeks.¹ The pathogenetic mechanisms of CU are still poorly understood. In a variable proportion (30–60%) of patients with active disease the intradermal injection of autologous serum (ASST, autologous serum skin test) causes a wheal-and-flare reaction^{1–5} and the serum from some CU patients is able to induce histamine release from cultured basophils of healthy subjects (HRA, histamine release assay). Both phenomena have been ascribed to circulating IgG specific for the high affinity IgE receptor FcεRI present on mast cells and basophils or for IgE.^{1–9} However, several aspects remain contradictory, and some evidence suggests that ASST and HRA look into distinct aspects of the disease and do not invariably indicate the presence of autoantibodies.

About 50% of patients with CU are positive on ASST,^{2–5,10} but sera from only 1/2 of them are able to induce histamine release from cultured basophils *in vitro*.^{1,5,10–13} We recently observed that the sensitivity of *in vitro* assays is much increased if both mast cells and basophils from several donors are used,¹⁴ suggesting a wide variability in the response of cells from different donors.

Serum from patients negative on ASST does not induce any histamine release *in vitro*,¹⁰ although these subjects do not show any clinical and/or pathological difference from ASST-positive/HRA-positive patients.

Histamine release induced by purified anti-FcεRI IgG *in vitro* is markedly enhanced by complement,^{9,15–17} whereas decomplexation does not influence the result of the ASST.^{10,13,18}

Finally, IgG-depleted autologous sera from patients with CU maintain their ability to induce wheal-and-flare

From ^aAmbulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano (MI); ^bUnità Operativa di Medicina Interna 2, Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Fondazione IRCCS, Milano; ^cUnità di Allergologia, Immunologia Clinica e Reumatologia, IRCCS Istituto Auxologico, Milano; and ^dDipartimento di Medicina Interna, Università di Milano.

Received for publication October 18, 2005; revised December 21, 2005; accepted for publication December 22, 2005.

Reprint requests: Riccardo Asero, MD, Ambulatorio di Allergologia, Clinica San Carlo, Via Ospedale 21, 20037 Paderno Dugnano (MI), Italy. E-mail: r.asero@libero.it.

0091-6749/\$32.00

© 2006 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.12.1343

Abbreviations used

CU: Chronic urticaria
 ASST: Autologous serum skin test
 APST: Autologous plasma skin test
 F₁₊₂: Prothrombin fragment 1+2
 HRA: Histamine release assay

reactions on intradermal injection,¹³ because ASST was detecting factors other than anti-FcεRI IgG.

Autologous serum skin test has become the easiest way to diagnose autoreactive CU in the clinical practice,¹⁹ but some authors have questioned the specificity of this test, suggesting that it might produce false-positive results because of the generation of large quantities of bradykinin during the clotting process²⁰ and the direct cleavage of C5 by tryptaselike plasma proteases secreted by neutrophils.²¹ If this were the case, intradermal testing with autologous plasma (APST, autologous plasma skin test) instead of autologous serum would be expected to result in a better specificity of *in vivo* assays (ie, in a reduced frequency of positive tests). Previous studies showed that heparin-anticoagulated plasma cannot be used because heparin inhibits degranulation of mast cells and basophils both *in vivo*^{10,22} and *in vitro*.¹⁰

The first aim of the current study was to investigate skin autoreactivity by using plasma anticoagulated with substances other than heparin (APST) and compare it with ASST in patients with CU. Second, because experimental and clinical data indicate that (1) thrombin, the final enzyme of the coagulation cascade, increases vascular permeability,^{23,24} (2) thrombin generation occurs during attacks of angioedema in patients with C1-inhibitor deficiency,²⁵ (3) thrombin injection can induce urticarial reactions,²⁶ and (4) both heparin (which highly increases the antithrombin activity in plasma) and oral anticoagulant therapy can be effective in the treatment of CU,^{27,28} we evaluated thrombin generation in plasma of our patients with CU by measuring prothrombin fragment 1+2 (F₁₊₂), inactive fragments considered to be a reliable marker of thrombin generation *in vivo*.²⁹

METHODS

Patients

Ninety-six consecutive adult patients (male:female, 24:72; age range, 18–70 years) with CU seen at the allergy department of the Clinica San Carlo (Paderno Dugnano, Italy) were studied. CU was diagnosed on the basis of the appearance of continuous or recurrent hives with or without angioedema for more than 6 weeks.¹ Patients with physical urticaria were excluded. Urticarial activity was estimated according to the number of wheals present at the time when blood samples from these 28 patients were collected as previously described³⁰: 1 to 10 small (<3 cm in diameter) wheals = grade 1 (slight); 10 to 50 small wheals or 1 to 10 large wheals = grade 2 (moderate); >50 small wheals or >10 large wheals = grade 3 (severe); virtually covered with wheals = grade 4 (very severe).

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, and all subjects gave their informed consent before participation.

Skin tests

After antihistamine treatment (cetirizine 10 mg, loratadine 10 mg, or ebastine 20 mg daily in all cases) had been stopped for at least 5 days, all patients underwent intradermal testing with 0.05 mL of both sterile autologous serum (ASST) and saline as negative control. Seventy-one patients underwent intradermal test with Na citrate–anticoagulated plasma (APST–Na citrate, 0.125 mol/L of Na citrate). Serum and plasma samples were centrifuged after 15 minutes at 1250g for 3 minutes and immediately used for intradermal tests. All intradermal tests (serum, plasma, and negative control) were performed and read at the same time in all patients. A skin prick test with histamine 10 mg/mL was used as positive control. Readings were taken at 30 minutes; only an unequivocal wheal-and-flare reaction with a wheal diameter of at least 3 mm in the absence of any reaction to saline was considered a positive response. Ten atopic subjects and 5 subjects with contact dermatitis, all without a history of chronic urticaria, underwent APST–Na citrate as control.

F₁₊₂ measurement

Na citrate–anticoagulated plasmas from 28 patients with CU and from 27 age-matched and sex-matched normal subjects were stored into plastic cones at –20°C until prothrombin fragment F₁₊₂.^{31,32} was measured by a sandwich ELISA (Enzygnost F₁₊₂; Behring Diagnostics GmbH, Frankfurt, Germany). Intra-assay and interassay coefficients of variation were 5% and 8%, respectively.

Statistics

Means were compared by 2-tailed Student *t* test. Probability (*P*) values less than 5% were considered statistically significant. Correlation between urticaria severity and F₁₊₂ plasma levels was assessed; an *r* value corresponding to a probability <5% was considered significant.

RESULTS

Skin tests

No patient reacted on the intradermal injection of saline, and skin prick test with histamine scored positive in all cases. Altogether, 51 of 96 (53%) patients scored positive on ASST.

We first used K₂EDTA as anticoagulant to test plasma skin reactivity. Of 25 patients submitted to intradermal testing with K₂EDTA-anticoagulated plasma, 24 (96%) scored positive. To assess the specificity of skin reactions induced by autologous plasma, 11 patients (4 negative and 7 positive on ASST) underwent further intradermal tests using saline containing 1 mg/mL K₂EDTA; this solution induced a wheal-and-flare reaction in all 11 cases. In addition, in 1 patient, the intradermal tests performed with different concentrations of K₂EDTA in saline (obtained adding different amounts of saline to sterile tubes containing K₂EDTA) showed that the intensity of the wheal-and-flare reaction was directly related to the concentration of K₂EDTA. Thus K₂EDTA induced nonspecific reactions and was no longer used to detect patients' autoreactivity.

Sixty-one of 71 (86%) patients scored positive on APST–Na citrate. The skin test with APST–Na citrate was

TABLE I. F_{1+2} measurement

All patients (n = 28)	3.07 [3.36]
ASST ⁺ /APST ⁺ patients (n = 19)	3.89 [3.68]
ASST ⁻ /APST ⁺ patients (n = 9)	1.33 [1.64]
Normal controls (n = 27)	0.80 [0.34]

Figures in square brackets are SD.

positive in 21 of 30 (70%) patients who scored negative on ASST and in 40 of 41 (98%) ASST-positive patients. In ASST-positive patients, the mean diameter of the wheal-and-flare area induced by autologous plasma exceeded that induced by autologous serum in 21 of 41 cases; in 19 cases, the wheal-and-flare area induced by autologous serum and plasma was equivalent, whereas in 1 case, the APST was negative. Altogether, of 10 patients negative on APST–Na citrate, 9 were negative on ASST as well, and only 1 was positive on ASST. To assess the specificity of skin reactions induced by autologous plasma, 10 patients (5 and 8 of whom were positive on ASST and APST–Na citrate, respectively) underwent further intradermal tests using Na citrate 0.125 mol/L in saline; no patient reacted to this solution. No skin reactivity was observed in 10 control subjects with allergic rhinitis but no history of urticaria on APST–Na citrate, or in the 5 control subjects with contact dermatitis.

F_{1+2} measurement

The results of F_{1+2} measurements in patients and normal controls are shown in Table I. On average, patients with CU showed a significantly higher plasma concentration of F_{1+2} than normal subjects (3.06 [SD 3.36] vs 0.80 [0.34]; $P < .001$). Interestingly, mean plasma F_{1+2} levels were higher in ASST⁺/APST⁺ than in ASST⁻/APST⁺ patients. Probably a result of the limited number of observations, this difference did not reach statistical significance (3.89 [SD 3.68] vs 1.33 [1.64]; $P = .058$; Fig 1). There was a statistically significant relationship between plasma F_{1+2} levels and severity of urticaria at the time blood samples were collected ($r = 0.37$; $P < .05$). The mean (SD) plasma F_{1+2} levels in patients with slight (n = 14), moderate (n = 8), or severe (n = 6) urticaria were 1.98 (2.78), 3.48 (3.78), and 5.04 (3.55), respectively (Fig 2).

DISCUSSION

The results of the current study seem to open some new insights into the pathogenesis of CU. The large majority of patients studied here, including many that would have been classified as having an idiopathic disease, scored positive on APST–Na citrate. The fact that APST–Na citrate was negative in all control subjects suggests that the wheal-and-flare reactions observed were specifically associated with CU. Our findings seem to rule out the possibility that ASST produces nonspecific results because of the generation of bradykinin and C5a during the clotting process, as suggested by some researchers.^{20,21} In fact, if this were the case, several patients would have

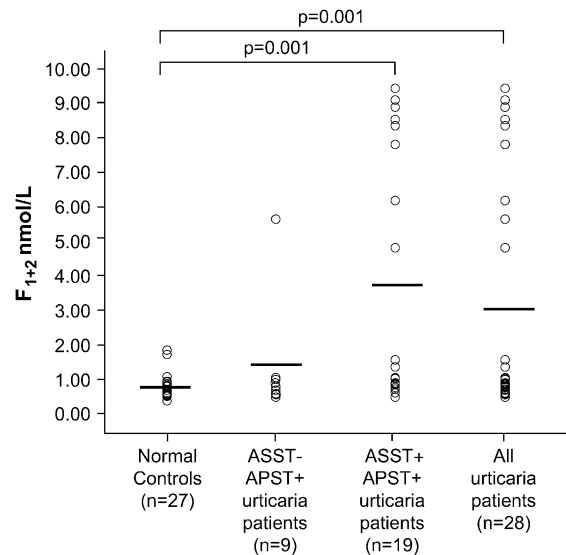


FIG 1. Plasma levels of prothrombin fragment F_{1+2} in normal controls and in patients with CU together and divided into different subsets according to skin reactivity to autologous serum and plasma. Results are expressed as nmol/L, and horizontal lines represent the mean values. F_{1+2} plasma levels were increased in patients with chronic urticaria, particularly in those with positive ASST.

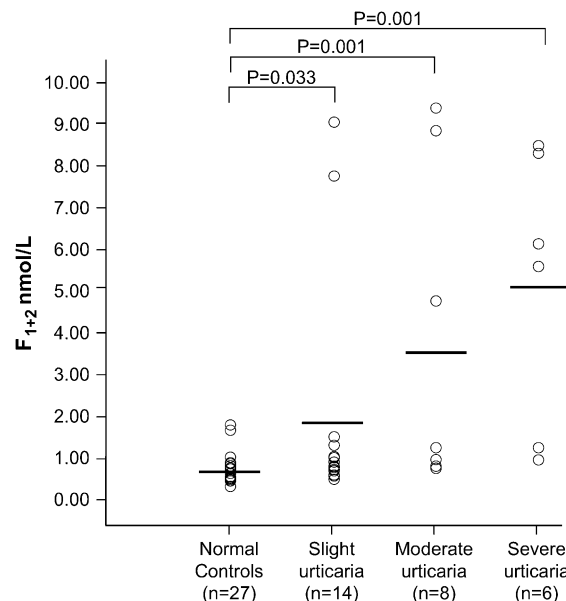


FIG 2. Plasma levels of prothrombin fragment F_{1+2} in normal controls and in patients with chronic urticaria divided into different subsets according to the severity of the disease (slight, moderate, and severe). Results are expressed as nmol/L, and horizontal lines represent the mean values. F_{1+2} plasma levels tended to parallel the severity of the disease.

scored negative on APST and positive on ASST, but we observed exactly the opposite situation.

Nevertheless, serum and Na citrate plasma samples from patients with CU showed a similar *in vitro* histamine-releasing activity on normal donors' basophils (unpublished

observations). This suggests that *in vitro* histamine-releasing activity on basophils does not entirely reflect what occurs *in vivo*. The striking difference between ASST and APST results pointed to a possible role by platelet-derived or clotting factors in the skin reactions. To assess this possibility, we measured plasma levels of F_{1+2} , a polypeptide of about 34 kD that is released into the circulation during the activation of prothrombin to thrombin by factor Xa, and observed that this was significantly increased in patients with CU. Interestingly, F_{1+2} plasma levels were related to urticaria severity at the time plasma was collected. This strongly suggests that CU is associated with the generation of thrombin, and that the severity of the disease is paralleled by the amount of thrombin generated. Thrombin is a serine protease involved in hemostasis as well as in vessel wound healing, revascularization, and tissue remodeling,³³ and exerts most of its effects through protease activated receptors.³⁴ There are several observations that make the findings of thrombin generation in CU all but surprising. One relevant effect of thrombin is edema through an increase in vascular permeability,^{35,36} an effect that is much reduced by antihistamines and totally abolished if mast cell granules are depleted.³⁶ Thrombin is able to trigger mast cell degranulation³⁷ and may activate protease activated receptor 1 on mast cells.³⁸ In some mast cell populations, the response to thrombin is equipotent with FcεRI-mediated activation.³⁸ Conversely, mast cells are able to convert prothrombin to thrombin by the release of tryptase.³⁹ Interestingly, it has been shown that in C1-inhibitor deficiency, both hereditary and acquired, acute attacks are associated with the generation of thrombin.²⁵ Further, heparin has been shown to inhibit the wheal-and-flare reaction on ASST^{10,22}; heparin is an activator of antithrombin-III, a plasma protein that binds irreversibly to some serine protease enzymes (factors XIa, IXa, Xa, VIIa, and thrombin) and blocks their activity. Finally, warfarin, an anticoagulant drug, induced a dramatic response in some patients with CU unresponsive to antihistamines in a double-blind, placebo-controlled study.²⁸ Oral anticoagulants inhibit the vitamin K-dependent γ-carboxylation of coagulation factors (II [prothrombin], VII, IX, and X); this results in the synthesis of biologically inactive forms of these coagulation proteins. It is interesting to note that a reduction of F_{1+2} plasma levels has been documented during oral anticoagulant treatment,⁴⁰ indicating a reduction of thrombin generation.

Although our findings show a relationship between positive ASST or urticaria severity and F_{1+2} levels (ie, the amount of thrombin generated), not all aspects are clear. Because of its extremely short half-life, it is unlikely that the wheal-and-flare reaction observed on autologous intradermal tests is caused by thrombin itself, and similar vasoactive effects by F_{1+2} have not been demonstrated.

Our findings with plasma anticoagulated with K_2 EDTA were unexpected. EDTA has been shown to block histamine release from mast cells,⁴¹ and in a previous study of CU, Fagiolo et al²² did not note any substantial difference in skin response to autologous serum or EDTA-anticoagulated plasma, nor did they observe any skin

reactivity to EDTA-containing saline in patients with CIU. From that study, it is not clear whether EDTA was used as such, as K_2 EDTA, or as Na_2 EDTA. The use of EDTA rather than K_2 EDTA salt might explain the striking differences between the findings of that study and of the current one, because potassium has been shown to induce histamine release from mast cells.⁴²

In conclusion, the current study demonstrates that the large majority of patients with CU are positive on intradermal testing with autologous plasma anticoagulated with Na citrate and that this skin test is specific. It also suggests that results of skin testing with both autologous plasma and serum is only partially associated with the presence of circulating antibodies to FcεRI or to IgE. Finally, it shows that CU is associated with the generation of thrombin, a serine protease able to activate mast cells and to cause relevant increase in permeability of endothelium.

We thank Dr Paolo Paparella, Laboratorio di Analisi, Clinica San Carlo, Paderno Dugnano, for providing plasma from normal subjects and for the useful discussions.

REFERENCES

- Greaves M. Chronic urticaria. *J Allergy Clin Immunol* 2000;105:664-72.
- Grattan CE, Wallington TB, Warin RP, Kennedy CT, Bradfield JW. A serological mediator in chronic idiopathic urticaria: a clinical, immunological and histological evaluation. *Br J Dermatol* 1986;114:583-90.
- Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan AP. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J Invest Dermatol* 1988;90:213-7.
- Grattan CEH, Francis DM, Hide M, Greaves MW. Detection of circulating histamine-releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 1991;21:695-704.
- Greaves MW. Chronic urticaria. *N Eng J Med* 1995;332:1767-72.
- Hide M, Francis DM, Grattan CEH, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Eng J Med* 1993;328:1599-604.
- Tong LJ, Balakrishnan G, Kochan JP, Kinet JP, Kaplan AP. Assessment of autoimmunity in patients with chronic urticaria. *J Allergy Clin Immunol* 1997;99:461-5.
- Fiebigler E, Maurer D, Holub H, Reininger B, Hartmann B, Woisetschlager M, et al. Serum IgG autoantibodies directed against the alpha chain of FcεRI, a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J Clin Invest* 1995;96:2606-12.
- Zweiman B, Valenzano M, Atkins PC, Tanus T, Getsy JA. Characteristics of histamine-releasing activity in patients with chronic idiopathic urticaria. *J Allergy Clin Immunol* 1996;98:89-98.
- Asero R, Tedeschi A, Lorini M, Salimbeni R, Zanoletti T, Miodonna A. Chronic urticaria: novel clinical and serological aspects. *Clin Exp Allergy* 2001;31:1105-10.
- Sabroe RA, Francis DM, Barr RM, Kobza-Black A, Greaves MW. Anti-FcεRI autoantibodies and basophil histamine releasability in chronic idiopathic urticaria. *J Allergy Clin Immunol* 1998;102:651-8.
- Ferrer M, Kinet JP, Kaplan AP. Comparative studies of functional and binding assays for IgG and anti-FcεRI (alpha subunit) in chronic urticaria. *J Allergy Clin Immunol* 1998;101:672-6.
- Fagiolo U, Krichek F, Ruf C, Peserico A, Amadori A, Cancian M. Effects of complement inactivation and IgG depletion on skin reactivity to autologous serum in chronic idiopathic urticaria. *J Allergy Clin Immunol* 2000;106:567-72.
- Asero R, Lorini M, Chong SU, Zuberbier T, Tedeschi A. Assessment of histamine-releasing activity of sera from patients with chronic urticaria showing positive autologous serum skin test on human basophils and mast cells. *Clin Exp Allergy* 2004;34:1111-4.
- Ferrer M, Nazakawa K, Kaplan AP. Complement-dependence of histamine release in chronic urticaria. *J Allergy Clin Immunol* 1999;104:169-72.

16. Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol* 2001;107:1056-62.
17. Kikuchi Y, Kaplan AP. A role for C5a in augmenting IgG-dependent histamine release from basophils in chronic urticaria. *J Allergy Clin Immunol* 2002;109:114-8.
18. Grattan CE, Hamon CG, Cowan MA, Leeming RJ. Preliminary identification of a low molecular weight serological mediator in chronic idiopathic urticaria. *Br J Dermatol* 1988;119:179-83.
19. Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza Black A, Greaves MW, et al. The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999;140:446-52.
20. Kaplan AP, Joseph K, Shibayama Y, Nakazawa Y, Ghebrehivert B, Reddigari S, et al. Bradykinin formation: plasma and tissue pathways and cellular interactions. In Gallin J, Snyderman R, editors. *Inflammation: basic principles and clinical correlates*. 3rd ed. Philadelphia: Lippincott, Williams and Wilkins; 1999. p. 331-47.
21. Ward PA, Hill JH. C5 chemotactic fragments produced by an enzyme in lysosomal granules of neutrophils. *J Immunol* 1970;104:535-43.
22. Fagiolo U, Cancian M, Bertollo L, Peserico A, Amadori A. Inhibitory effect of heparin on skin reactivity to autologous serum in chronic idiopathic urticaria. *J Allergy Clin Immunol* 1999;103:1143-7.
23. Garcia JG, Siflinger-Birnboim A, Bizios R, Del Vecchio PJ, Fenton JW 2nd, Malik AB. Thrombin-induced increase in albumin permeability across the endothelium. *J Cell Physiol* 1986;128:96-104.
24. DeMichele MA, Moon DG, Fenton JW 2nd, Minnear FL. Thrombin's enzymatic activity increases permeability of endothelial cell monolayers. *J Appl Physiol* 1990;69:1599-606.
25. Cugno M, Cicardi M, Bottasso B, Coppola R, Paonessa R, Mannucci PM, et al. Activation of the coagulation cascade in C1-inhibitor deficiencies. *Blood* 1997;89:3213-8.
26. Sheldon PJ, Oglevie SB, Kaplan LA. Prolonged generalized urticarial reaction after percutaneous thrombin injection for treatment of a femoral artery pseudoaneurysm. *J Vasc Interv Radiol* 2000;11:759-61.
27. Chua SL, Gibbs S. Chronic urticaria responding to subcutaneous heparin sodium. *Br J Dermatol* 2005;153:216-7.
28. Parslew R, Pryce D, Ashworth J, Friedmann PS. Warfarin treatment of chronic idiopathic urticaria and angio-oedema. *Clin Exp Allergy* 2000;30:1161-5.
29. Bauer KA, Weitz JI. Laboratory markers of coagulation and fibrinolysis. In Colman RW, Hirsh J, Marder VJ, Clowes AW, George JN, editors. *Hemostasis and thrombosis: basic principles and clinical practice*. Philadelphia: JB Lippincott; 2001. p. 1113-29.
30. Sabroe RA, Fiebiger E, Francis DM, Maurer D, Seed PT, Grattan CEH, et al. Classification of anti-FcεRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002;110:492-9.
31. Pelzer H, Schwart A, Stuber W. Determination of human prothrombin activation fragment 1+2 in plasma with an antibody against a synthetic peptide. *Thromb Haemost* 1991;65:153-9.
32. Hursting MJ, Butman BT, Steiner JP, Moore BM, Planck MC, Szewczyk KM, et al. Monoclonal antibodies specific for prothrombin fragment 1.2 and their use in a quantitative enzyme-linked immunosorbent assay. *Clin Chem* 1993;39:583-91.
33. Goldsack NR, Chambers RC, Dabbagh K, Laurent GJ. Thrombin. *Int J Biochem Cell Biol* 1998;30:641-9.
34. Coughlin SR. How the protease thrombin talks to cells. *Proc Natl Acad Sci U S A* 1999;96:11023-7.
35. Schaeffer RC, Gong F, Bitrick MS, Smith TL. Thrombin and bradykinin initiate discrete endothelial solute permeability mechanisms. *Am J Physiol* 1993;264:1798-809.
36. Cirino G, Cicala C, Bucci MR, Sorrentino L, Maranganore JM, Stone SR. Thrombin functions as an inflammatory mediator through activation of its receptors. *J Exp Med* 1996;183:821-7.
37. Razin E, Marx G. Thrombin-induced degranulation of cultured bone marrow-derived mast cells. *J Immunol* 1984;133:3282-5.
38. Vliagoftis H. Thrombin induces mast cell adhesion to fibronectin: evidence for the involvement of protease-activated receptor-1. *J Immunol* 2002;169:4551-8.
39. Kido H, Fukusen N, Katunuma N, Morita T, Iwanaga S. Trypsin from rat mast cells converts bovine prothrombin to thrombin. *Biochem Biophys Res Commun* 1985;132:613-9.
40. Bruhn HD, Liebsch J, Wagner C. Documentation of hypocoagulability by measurement of prothrombin fragment F 1 + 2 when introducing oral anticoagulant therapy. *Thromb Res* 1992;68:317-9.
41. West GB. Ethylene diamine tetraacetic acid and histamine release from rat mast cells. *Int Arch Allergy Appl Immunol* 1982;68:399-401.
42. Nemeth A, Magyar P, Herczeg R, Huszti Z. Potassium-induced histamine release from mast cells and its inhibition by ketotifen. *Agents Actions* 1987;20:149-52.