

ORIGINAL ARTICLE

Activation of blood coagulation in plasma from chronic urticaria patients with negative autologous plasma skin test

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Abstract

Background Skin reactivity to the intradermal injection of autologous serum (autologous serum skin test – ASST) and/or plasma (autologous plasma skin test – APST) is thought to identify chronic urticaria (CU) patients with an autoimmune/autoreactive disease. Immune-mediated inflammation and coagulation are strictly linked, and coagulation activation has been described in CU patients as shown by the elevation of plasma prothrombin fragment F1 + 2 and, in severe cases, of D-dimer as well.

Objective The aim of this study was to evaluate whether the coagulation cascade is activated in APST-negative CU patients as it has been described in CU patients with an autoreactive disease.

Methods A total of 43 adults with CU (M/F 15/28; mean age 43.5 years; 16 APST-negative patients and 27 APST-positive) and 30 healthy subjects were studied. Prothrombin fragment F1 + 2, D-dimer and C-reactive protein (CRP) plasma levels were measured by ELISA.

Results Prothrombin fragment F1 + 2 and D-dimer were elevated in seven of 16 APST-negative CU patients. The activation of the coagulation cascade was associated with disease severity. Men were more prevalent in idiopathic than in autoreactive CU patients (M/F: 10/6 vs. 5/22; $P < 0.001$). In patients with APST-negative CU, mean F1 + 2 level [242.8 ± 33.7 pmol/L (ESM)] was higher than in normal controls (151.8 ± 9.09 pmol/L; $P = 0.002$) but lower than in autoreactive patients (526.2 ± 97.8 pmol/L; $P = 0.05$). Similarly, mean D-dimer level was higher than in normal controls (484.2 ± 148.3 ng/mL vs. 229.5 ± 16.7 ng/mL; $P = 0.03$) but lower than in autoreactive patients (1142.2 ± 317.4 ng/mL; $P = 0.05$). In contrast, mean CRP was lower than in autoreactive patients (1.06 ± 0.32 µg/mL vs. 3.09 ± 0.74 µg/mL; $P = 0.02$) but not different from normal subjects (0.78 ± 0.09 µg/mL; NS).

Conclusion Autologous plasma skin test-negative CU prevails in men; in these patients the coagulation cascade is activated although with a lower intensity than in patients with autoreactive disease.

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Keywords

chronic urticaria, autologous plasma skin test, coagulation

Conflict of interest

None declared.

Introduction

Chronic ordinary urticaria (CU) is a skin disorder characterized by the recurrent eruption of short-lived wheals with or without angioedema for more than 6 weeks¹ that may heavily impair the quality of life.² Studies performed during the last 15 years have shown that the pathogenesis of this disease is autoimmune in a

proportion (25–35%) of cases, being associated with functionally active autoantibodies to IgE or to the high affinity IgE receptor, FcεRI.^{3–8} A larger proportion (about 50%, a proportion that includes subjects with an autoimmune disease) of patients with CU are characterized by an autoreactive state, as the intradermal injection of autologous serum (ASST, autologous serum skin test)

elicits a wheal-and-flare skin reaction.^{1,9} We were recently able to show that the proportion of patients showing an autoreactive disease increases to >80% if autologous citrated plasma instead of autologous serum is injected.¹⁰ The following studies demonstrated the activation of the coagulation cascade in CU. One study found thrombin generation, as shown by elevated plasma levels of prothrombin fragment $F1 + 2$;¹⁰ subsequent ones showed that thrombin generation stems from the activation of the extrinsic pathway of coagulation cascade,¹¹ and that in patients with a severe disease such activation may be intense enough to lead to an elevation of plasma levels of D-dimer.¹² Finally, we recently demonstrated that in CU tissue factor is hyper-expressed by eosinophils present in the inflammatory infiltrate of CU skin lesions.¹³ In CU patients, the activation of the coagulation cascade parallels disease activity, and a marked increase of plasmatic markers of thrombin generation and fibrinolysis can be observed during severe exacerbations of the disease.¹² The activation of coagulation and fibrinolysis decrease to complete normalization during remission phases of the disease.^{12,14} Whether the activation of coagulation/fibrinolysis plays a pivotal role in the pathophysiology or acts simply as an amplification system is still to be defined. In experimental models, thrombin has been shown to induce oedema through an increase in vascular permeability^{15,16} by a direct effect on endothelial cells¹⁷ and indirectly by a thrombin-related release of inflammatory mediators, and to trigger mast cell degranulation;¹⁸ as a consequence, it has been logical to hypothesize that coagulation cascade was involved in the wheal-and-flare reaction induced by autologous plasma.

As some CU patients score negative on APST and seem to have an 'idiopathic' disease, we studied the coagulation cascade in this subset to assess whether they show the same signs of activation of the coagulation cascade as subjects with an 'autoreactive' disease.

Patients and methods

Subjects

A total of 43 adults with chronic urticaria (CU; M/F 15/28; mean age 43.5 years, range 17–72 years) seen at the allergy centres of Paderno Dugnano and Milano were studied. The diagnosis of CU was based on the presence of continuous or recurrent hives with or without angio-oedema for more than 6 weeks. Patients with physical urticaria were excluded. All subjects underwent an intradermal skin test using 0.05 mL of citrated autologous plasma (APST, autologous plasma skin test) as previously described¹⁰ and, based

on the APST outcome, were divided into APST-negative patients ($n = 16$) and 'autoreactive', APST-positive controls ($n = 27$).

At the time of blood sampling, both patients and autoreactive controls had active urticaria, and disease activity was graded from 1 to 6 (mild, moderate or severe) according to the recently published EAACI/GA2LEN Urticaria activity score¹⁹ as reported in Table 1.

All patients gave an informed consent to APST and blood collection. In total 30 healthy subjects (M/F 15/15, mean age 49.7 years, range 35–76 years) were chosen as control. The local Institutional Review Board approved the study.

Prothrombin fragment 1 + 2 and D-dimer measurement

Prothrombin fragment F_{1+2} was measured by a sandwich ELISA (Enzygnost F_{1+2} ; Behring Diagnostics GmbH, Frankfurt, Germany). Intra-assay and inter-assay CVs were 5% and 8% respectively. Values below 229 pmol/L were considered normal.

D-dimer plasma levels were measured by ELISA (Enzygnost D-dimer; Behring Diagnostics GmbH, Frankfurt, Germany) according to manufacturer's instructions. The intra- and inter-assay CVs were 10% and 15% respectively. Based on the calibration standards provided by the manufacturer, levels <278 ng/mL were considered negative. The positive-low and positive-high control standards provided with the batch used scored 395 and 770 ng/mL respectively.

C-reactive protein determination

C-reactive protein (CRP) plasma concentration was measured using a sandwich enzyme immunoassay (Zymutest CRP, Hyphen BioMed, Neuville-sur-Oise, France). Intra- and inter-assay variability was lower than 11%.

Statistics

Proportions were compared by chi-squared test with Yates' corrections. Means were compared by two-tailed Student's *t*-test for unpaired data with Welch's correction, after log transformation of data. Correlations between disease severity and $F1 + 2$, D-dimer and CRP plasma levels were assessed by Pearson test after log transformation of data. Probability (*P*) values <5% were considered statistically significant.

Results

The clinical features of the 16 patients with APST-negative CU are shown in Table 2. Seven patients showed elevated plasma levels of

Table 1 Severity score of chronic urticaria according to EAACI/GA2LEN guidelines

	Wheals	Pruritus
Score 0	None	None
Score 1 (Mild)	<20 wheals/24 h	Present but not annoying or troublesome
Score 2 (Moderate)	20–50 wheals/24 h	Troublesome but does not interfere with normal daily activity or sleep
Score 3 (Intense)	>50 wheals/24 h or large confluent areas of wheals	Severe pruritus, interfering with normal daily activity or sleep

Table 2 Clinical features and plasma levels of prothrombin fragment $F1 + 2$, D-dimer and C-reactive protein (CRP) in patients with APST-negative chronic urticaria

Patient	Gender	Age	Severity	$F1 + 2$	D-dimer	CRP
1	F	29	2	265.57	253.54	3.568
2	M	48	2	154.62	374.03	0.535
3	M	68	3	157.31	466.72	0.821
4	F	36	1	358.11	155.44	0.53
5	M	37	2	138.19	771.17	0.832
6	F	44	2	220.35	278.28	0.229
7	F	50	2	120.46	233.78	0.437
8	M	31	2	246.08	885.17	0.349
9	F	37	2	194.03	147.51	1.651
10	M	19	2	90.13	133.59	0.291
11	F	48	5	636.48	2478.92	0.435
12	M	54	2	211.14	154.25	0.417
13	M	39	2	156.7	173.66	0.39
14	M	75	5	408.44	873.95	4.783
15	M	61	3	252.25	171.82	0.413
16	M	64	3	275.54	194.84	1.201

prothrombin fragment $F1 + 2$; seven showed elevated D-dimer plasma levels. A significant correlation was found between disease severity score and $F1 + 2$ ($r = 0.685$, $P = 0.003$) and D-dimer ($r = 0.704$, $P = 0.002$) plasma levels. Conversely, the correlation between disease severity score and CRP ($r = 0.430$, $P = 0.097$) was not statistically significant.

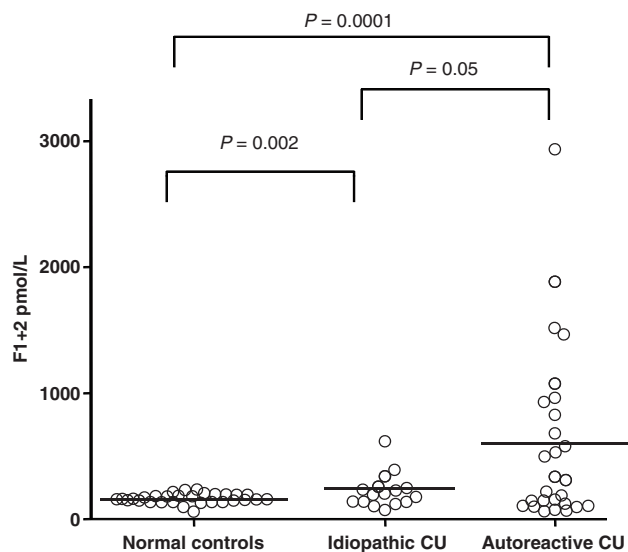


Figure 1 Plasma levels of prothrombin fragment $F1 + 2$ in patients with idiopathic urticaria, in patients with autoreactive urticaria and in normal controls. Results are expressed as pmol/L, and horizontal lines represent the mean values. $F1 + 2$ plasma levels were significantly higher in patients with chronic urticaria either idiopathic or autoreactive than in normal controls.

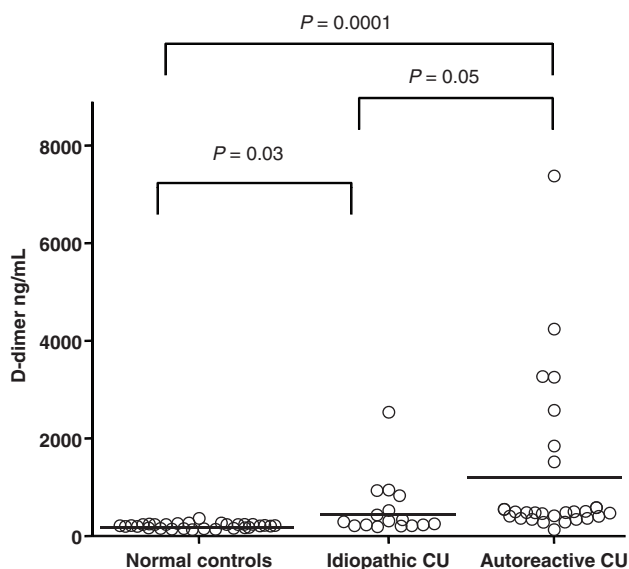


Figure 2 Plasma levels of D-dimer in patients with idiopathic chronic urticaria, in patients with autoreactive urticaria and in normal controls. Results are expressed as ng/mL, and horizontal lines represent the mean values. D-dimer plasma levels were significantly higher in patients with chronic urticaria either idiopathic or autoreactive than in normal controls.

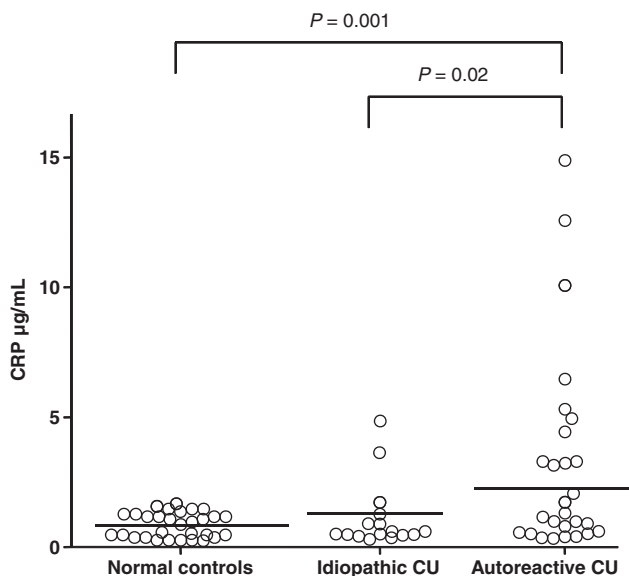


Figure 3 Plasma levels of C-reactive protein in patients with idiopathic chronic urticaria, in patients with autoreactive urticaria and in normal controls. Results are expressed as µg/mL, and horizontal lines represent the mean values. C-reactive protein plasma levels were significantly higher in patients with autoreactive chronic urticaria than in normal controls. No difference was found between patients with idiopathic chronic urticaria and normal controls.

Table 3 Comparison between APST-negative CU patients and controls

	APST neg.	APST pos.	Normal controls
N	16	27	30
Gender (M/F)	10/6*	5/22	15/15
Mean F1 + 2 levels – pmol/L (SD)	242.8 (134.9)†,‡	615.1 (690.3)	151.8 (49.8)
Mean D-dimer – ng/mL (SD)	484.2 (593.1)†,§	1142.2 (1649.5)	249.3 (81.7)
Mean CRP – µg/mL (SD)	1.06 (1.29)†,¶	3.09 (3.84)	1.17 (1.44)

APST, autologous plasma skin test; CU, chronic urticaria.

* $P < 0.001$ by comparison with controls with autoreactive CU.

† $P < 0.05$ by comparison with controls with autoreactive CU.

‡ $P < 0.005$ by comparison with normal controls.

§ $P < 0.005$ by comparison with normal controls.

¶NS by comparison with normal controls.

Patients with APST-negative CU, controls with autoreactive CU and normal controls are compared in Table 3. Interestingly, patients with APST-negative CU showed a significantly higher prevalence of men than autoreactive controls (M/F ratio: 10/6 vs. 5/22; $P < 0.001$). The results of *in-vitro* measurements in patients and in both positive and negative controls are shown and compared in Figs 1–3. The mean prothrombin fragment F_{1+2} level in patients exceeded that found in normal controls [242.8 ± 33.7 pmol/L (ESM) vs. 151.8 ± 9.09 pmol/L; $P = 0.002$] but was significantly lower than that found in the 'autoreactive' control group (242.8 ± 33.7 pmol/L vs. 526.2 ± 97.8 pmol/L; $P = 0.05$). Similarly, the mean plasma level of D-dimer exceeded that of normal controls (484.2 ± 148.3 ng/mL vs. 229.5 ± 16.7 ng/mL; $P = 0.03$) but was significantly lower than autoreactive controls (484.2 ± 148.3 ng/mL vs. 1142.2 ± 317.4 ng/mL; $P = 0.05$). Finally, the mean CRP level in patients was significantly lower than in autoreactive CU controls (1.06 ± 0.32 µg/mL vs. 3.09 ± 0.74 µg/mL; $P = 0.02$) but did not differ significantly from normal controls (1.06 ± 0.32 µg/mL vs. 0.78 ± 0.09 µg/mL; NS).

Discussion

In this study, we investigated the coagulation cascade in patients with APST-negative CU. We investigated this particular subset of CU patients because in previous studies we demonstrated that CU patients whose serum contain functionally active antibodies usually score positive on ASST,⁹ and that patients positive on ASST score always positive on APST as well.¹⁰ Thus, to the best of our current knowledge, CU patients negative on APST should not have functionally active circulating autoantibodies to IgE or to the high affinity IgE receptor, FcεRI, nor have any evidence of circulating histamine-releasing factors.

This work demonstrates that in patients with APST-negative CU, the coagulation cascade is activated although to a lesser extent than in patients with autoreactive CU and that, similarly, such activation parallels disease severity.¹² This suggests that in CU, the coagulation system is activated irrespective of the subset of the disease and that it probably does not play an important role in the wheal-and-flare skin response to autologous plasma. Further, the

whole inflammatory mechanism seems to be activated at a lower level in patients negative on APST, as shown by the normal CRP levels found in this subset.

Another relevant finding, which confirms one previous observation,²⁰ is the association of APST-negative CU with male gender. Although it is difficult to explain the reasons for such gender difference, one has to take into account that the association of most autoimmune diseases with female gender is widely established. This holds true also for autoimmune thyroiditis that is frequently associated with CU. Similar findings were recently reported by Najib *et al.*,²¹ who observed that thyroid autoimmunity and positivity of serum basophil activation test occur more frequently in female than in male CU patients.

In conclusion, the activation of the coagulation cascade seems a potential amplifying mechanism in CU but is probably not the trigger of the disease. Further studies are needed to detect which circulating factors cause the skin reaction upon intradermal injection of autologous serum or plasma²² and/or induce mast cell degranulation in patients without any evidence of circulating autoantibodies.

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