

Letter to the Editor

Increased thrombin generation potential in patients with chronic spontaneous urticaria



Dear Editor

Chronic spontaneous urticaria (CSU) is a common skin disorder of unknown etiology characterized by spontaneously appearing wheals and pruritus for six weeks or longer. Recent studies have supported an autoimmune origin in a population of patients with CSU. Circulating autoantibodies against the high affinity IgE receptor (FcεRI) and IgE are able to cause mast cell degranulation.¹ On the other hand, the possible involvement of the blood coagulation system has emerged.² This notion was supported by moderate increases of several coagulation markers, such as prothrombin fragment 1+2 (PF1+2), fibrin degradation products (FDP) and D-dimer in correlation with clinical severity, and the efficacy of anti-coagulants in cases of CSU.³ However, conventional coagulation assays, such as activated partial thromboplastin time (APTT), that measure only the initiation time of clot formation do not reveal apparent abnormality in patients with CSU. Since many processes and proteins are involved in the coagulation, several global coagulation tests have been developed to assess the whole coagulation process.

We previously revealed the increase of intrinsic coagulation potential in CSU patients, by one of the global coagulation tests, the APTT clot waveform analysis, and suggested that the activation of intrinsic coagulation factors may cause mast cell degranulation through the activation of protease-activating receptor-2 (PAR-2).⁴ However, an increased coagulation potential shown in the assay may not necessarily reflect the enhancement of intermediate coagulation factors, because the assay assesses the entire coagulation process from the beginning to fibrinogen-to-fibrin conversion. We, therefore, cannot exclude possible contributions of significantly elevated levels of fibrinogen, which may reflect chronic inflammation in CSU, to the hypercoagulable pattern of the assay. To clarify this, we further analyzed coagulation potentials in CSU, using calibrated automated thrombography (CAT). This approach measures the conversion of prothrombin to thrombin without fibrin generation (Fig. 1 inset).⁵ We also investigated the association between thrombin generation potentials and severities of CSU as well as the involvement of an autoimmune mechanism shown by autologous serum skin test (ASST), an *in vivo* test to demonstrate wheal-inducing factors in a patient's serum and to assess autoreactivity.⁶

We recruited 15 normal healthy individuals and 36 patients with CSU, who were the same population examined in our previous report.⁴ The mean $\pm$ SD age of healthy individuals was 37.4 ± 14.0 years (range 27–80) and that of CSU patients was 47.0 ± 18.0 years (range 9–76). No significant age difference was seen between the two groups. All patients were diagnosed on the basis of the appearance of spontaneous wheals for more than 6 weeks. The severity of CSU was assessed and described in the medical record at the time of blood collection, and patients with CSU were divided into three groups based on an urticaria activity score⁷: 10 patients were classified as mild, 19 as moderate, and 7 as severe. ASST was performed on 25 patients: 10 patients were positive and 15 negative. All patients were treated with H1 antagonists. Twenty-five patients received leukotriene receptor antagonist, tranexamic acid, H2 receptor antagonists, and other second-line treatments according to the Japanese guidelines for the management of urticaria. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, and was approved by the Institutional Review Board at each institution. All subjects gave written informed consent.

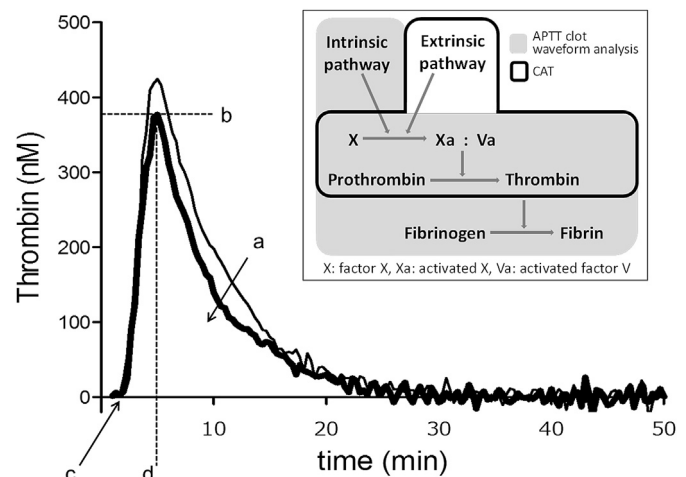


Fig. 1. A typical thrombin generation curve of healthy control (thick line) and patients with chronic spontaneous urticaria. Four parameters extracted from this assay: a, endogenous thrombin potential (ETP); b, peak height (Peak); c, lag time (Lagtime); d, time to peak (ttPeak). The inset shows coagulation process that is covered by calibrated automated thrombography (black border) and APTT clot formation analysis (halftone).

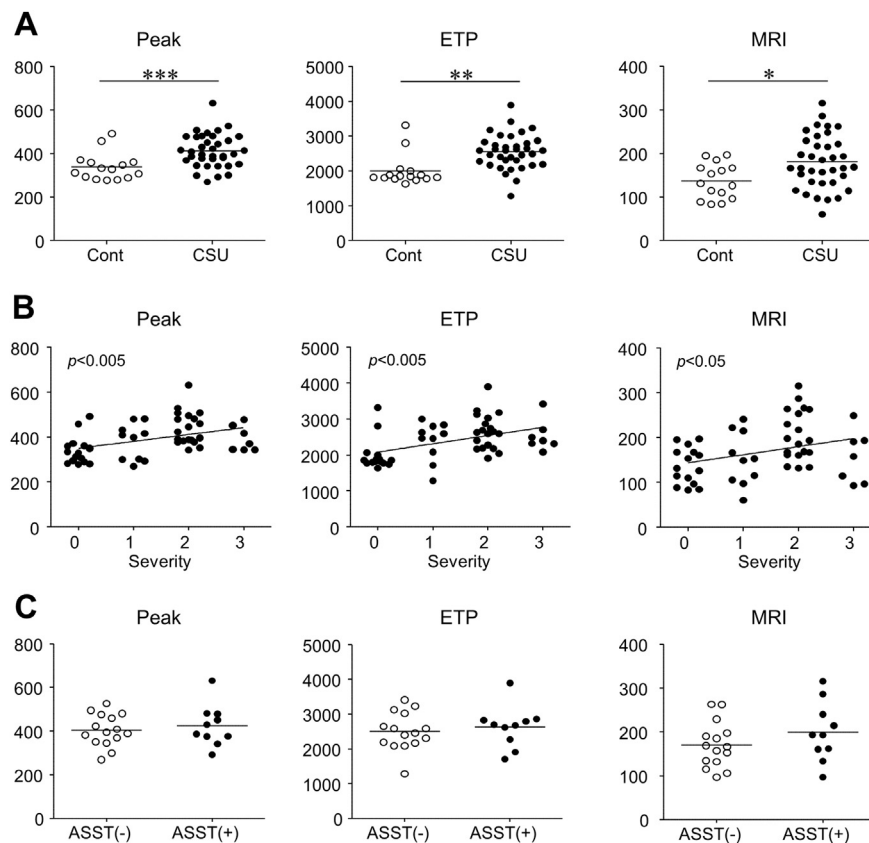


Fig. 2. Parameters of calibrated automated thrombography. Upper: parameters in healthy controls (open circles) and patients with CSU (filled circles). Bars indicate means. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Middle: correlations between severity of CSU and parameters. Lower: Parameters in ASST-negative ($n = 15$; open circles) and ASST-positive patients ($n = 10$; filled circles). Bars indicate means.

CAT does not measure only the total amount of produced thrombin, but also reveal the kinetics of thrombin generation and the peak activity in time course. The assay was performed in a microtiter plate fluorometer using dedicated software (Thrombinoscope). Briefly, coagulation in platelet-poor plasma was triggered by recalcification using 5 pM PPP-reagent (Thrombinoscope). The molar amount of thrombin was calculated from the signal of the wells and a calibration curve (Fig. 1). The total amount of thrombin activity, called the endogenous thrombin potential (ETP), was assessed as the area under the thrombin concentration–time curve. The program also calculates parameters including lag time (Lag-time), peak height (Peak), and time to peak (ttPeak). Mean Rate Index (MRI) was calculated by the formula $MRI = Peak / (ttPeak - Lagtime)$. Statistical analysis of differences was determined by the Mann–Whitney test. Correlations were calculated according to Spearman's rank correlation coefficient.

CAT revealed a significant elevation of Peak, ETP, and MRI in the patient group compared with the control group ($p < 0.001$, $p < 0.01$, $p < 0.05$, respectively) (Fig. 2A). A significant correlation was observed between the severity of CSU and each of CAT parameters [Peak ($p < 0.01$), ETP ($p < 0.001$), and MRI ($p < 0.05$)] (Fig. 2B). In CAT parameters, Peak describes the maximum thrombin activity and ETP indicates the total amount of thrombin that is generated throughout the reaction.^{8,9} MRI reflects the velocity of the propagation phase of thrombin generation.¹⁰ These key parameters showed a significant elevation in patients with CSU as well as a significant correlation with disease severities, which strongly suggested an increase of prompt coagulation

potential in patients with CSU regardless of the elevated levels of fibrinogen.

In our previous study, we showed that the amount of actually generated thrombin in patients with CSU was not sufficient for intravascular clotting. The levels of D-dimer and FDP that reflect the amount of actually generated thrombin were elevated but mostly remained within normal ranges. No significant elevation of the levels of PF 1+2 that directly reflect the amounts of actually generated thrombin was observed. These observations suggest that large-scale thrombin generation (so-called “thrombin burst”) that leads to fibrinogen cleavage and fibrin polymerization does not occur in patients with CSU. In the clinical setting, thrombotic events are not usually a complication of CSU. Furthermore, as CAT revealed no differences between ASST-positive and -negative groups in any parameters (Fig. 2C), the hypercoagulable state may develop independently of autoreactive mechanisms.

Taken together, coagulation potential is increased in CSU without the involvement of the elevated fibrinogen levels as well as autoreactive mechanisms. Subthreshold acceleration of intermediate phases of coagulation accompanied by small amount of thrombin generation may be critical in the pathogenesis of CSU. Our findings may lead to the development of new therapeutic strategies for CSU focusing on targeted inhibition of intermediate coagulation factors.

Conflict of interest

The authors have no conflict of interest to declare.

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