

REVIEW ARTICLE

Chronic urticaria and coagulation: pathophysiological and clinical aspects

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To cite this article: Tedeschi A, Kolkhir P, Asero R, Pogorelov D, Olisova O, Kochergin N, Cugno M. Chronic urticaria and coagulation: pathophysiological and clinical aspects. *Allergy* 2014; DOI: 10.1111/all.12389.

Keywords

chronic urticaria; coagulation; eosinophil; thrombin; tissue factor.

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Accepted for publication 4 February 2014

DOI:10.1111/all.12389

Edited by: Werner Aberer

Abstract

Chronic urticaria (CU) is a widespread skin disease, characterized by the recurrence of transient wheals and itch for more than 6 weeks. Besides autoimmune mechanisms, coagulation factors, in particular tissue factor and thrombin, might also participate in the disease pathophysiology. Tissue factor expressed by eosinophils can induce activation of blood coagulation generating thrombin which in turn can increase vascular permeability both directly, acting on endothelial cells, and indirectly, inducing degranulation of mast cells with release of histamine, as demonstrated in experimental models. D-dimer, a fibrin degradation product, generated following activation of the coagulation cascade and fibrinolysis, has been found to be increased during urticaria exacerbations; moreover, it has been proposed as a biomarker of severity and resistance to H1-antihistamines in CU patients. The possible role of coagulation in CU is also supported by case reports, case series and a small controlled study showing the efficacy of anticoagulant therapy in this disease. The purpose of this review was to summarize the available data on the possible contribution of coagulation to the pathophysiology of CU focusing on clinical aspects and possible future therapeutic developments.

Chronic urticaria (CU) is a widespread skin disease characterized by the recurrence of transient wheals and itch for more than 6 weeks. It is believed that over 50% of CU cases are accompanied by a deep subcutaneous and/or submucosal edema, called angioedema (AE; 1).

It is well known that the characteristic symptoms of urticaria appear following the activation of mast cells (MCs) and basophils in the skin by various stimuli. These cells release several biologically active substances, the most important of which is histamine. Histamine induces vasodilation, increases vascular permeability and stimulates sensory nerve endings. These effects lead to the appearance of erythema, wheals and itch (2). It is supposed that other biologically active substances may have similar effects. These include serotonin, C3a and C5a anaphylatoxins, platelet-activating factor (PAF), neuropeptides, and arachidonic acid metabolites (prostaglandin D2, leukotrienes C4, D4 and E4; 3).

Activation and degranulation of basophils and MCs may occur by immunological (formation of immune complexes,

complement activation, IgE cross-linking), nonimmunological (pseudoallergy, infection, or direct effect of agents on MCs), or mixed mechanisms. The mechanism and cause of urticaria remain unclear in many patients with CU.

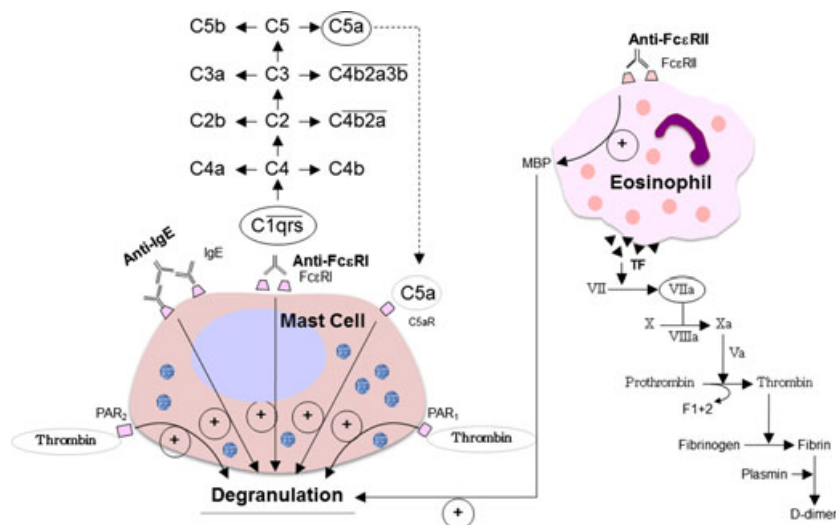
In recent years, attention was paid to several important aspects of the disease pathogenesis. It is believed that about 30–50% of patients have CU symptoms associated with autoimmune reactions and synthesis of autoantibodies against IgE (4) and/or the FcεRI α-subunit on MCs and basophils (autoimmune/autoimmune urticaria; 5). Furthermore, these patients show an increased frequency of HLA DRB1*04 (DR4) as it occurs in other autoimmune diseases (6). The role of coagulation cascade in the pathophysiology of urticaria has been described in recent studies (7–11). It is assumed that in patients with CU, there is a tight interplay between coagulation and inflammation which activate each other (11). The action of an appropriate stimulus on inflammatory cells, namely eosinophils, may trigger the expression of tissue factor (TF), which activates the extrinsic pathway of

The purpose of this review is to summarize the currently existing scientific data on the role of coagulation cascade in the development of CU. We carried out a search in PubMed using ‘urticaria’ and ‘coagulation’ as keywords, covering a period from 1950 to 2013.

It is known that TF, which activates the coagulation cascade, is present in an inactive form in the cytoplasm of various peripheral blood cells, such as monocytes, eosinophils, neutrophils and platelets (20, 21). In 2007 Moosbauer et al. (21) noted the leading role of activated eosinophils as an intravas-

It is not entirely clear how the process of eosinophil activation starts. Puccetti et al. (26) reported eosinophil activation by autoantibodies directed against the low-affinity IgE receptor (FcεRII) CD23 antigen. These autoantibodies have been detected in about 70% of CU patients and it is conceivable that they play a role in the pathogenesis of the disease. In fact, eosinophils activated by anti-FcεRII autoantibodies release the major basic protein (MBP) which in turn may cause MC degranulation (26). On the other hand, the activation of eosinophils may be secondary to the activation of MC by anti-FcεRI and anti-IgE autoantibodies or by other as yet unknown factors (27). Eosinophil activation and recruitment may be due to the action of mediators, cytokines and chemokines released by MC. These include IL-5, TNF-α, PAF and eotaxin (28–30; Fig. 1).

It is conceivable that the importance of the different cells involved in CU pathophysiology changes in different subsets of patients. In patients showing circulating anti-FcεRI and



affinity IgE receptor (FcεRII) releasing inflammatory mediators including the major basic protein (MBP) which in turn can induce MC degranulation. Activated eosinophils express tissue factor (TF) which is the main initiator of the coagulation cascade leading to thrombin formation. Thrombin in turn, as demonstrated in experimental models, may activate MCs by interaction with the protease-activated receptors (PAR) 1 and 2.

anti-IgE autoantibodies it is very likely that the main actor is the MC whereas in other patients showing only anti-FcεRII autoantibodies such role might be played by eosinophils (27). Finally, it cannot be excluded that T cells, which have been found in CU lesional skin, also play a relevant role (Fig. 2), possibly by activating MC via a cell-to-cell contact, as demonstrated in experimental models (31). Studies on animal models have shown that thrombin can induce MC degranulation with a potency similar to that of FcεRI-mediated activation (32). In a rat model, Dugina et al. (33) have demonstrated that the activation of MC via thrombin action on protease-activated receptors (PAR) is enhanced during inflammation. PAR is a new family of G protein-coupled receptors. There are four types of receptors in this family: PAR 1–4. PAR-2 agonists can cause the mobilization of

Ca²⁺, which promotes the release of histamine (33). MC express PAR-1 and PAR-2. The generated thrombin can activate MC via PAR-1, while TF + factor VIIa (FVIIa) and factor Va (FVa) + factor Xa (FXa) complexes act via PAR-2, leading to cell degranulation (34, 35). On the other hand, MC-derived tryptase can induce thrombin generation through a direct activation of prothrombin (36), thus generating an amplification loop. The first demonstration of coagulation activation in CU patients was provided by Asero et al. (37) in 2006, who found elevated levels of the coagulation marker F1 + 2. A year later, the same group described an activation of the extrinsic pathway of blood coagulation showing elevated levels of activated factor VII (FVIIa) and normal levels of activated factor XII (FXIIa; 38). The study conducted by Wang et al. (39) demonstrated that FVIIa

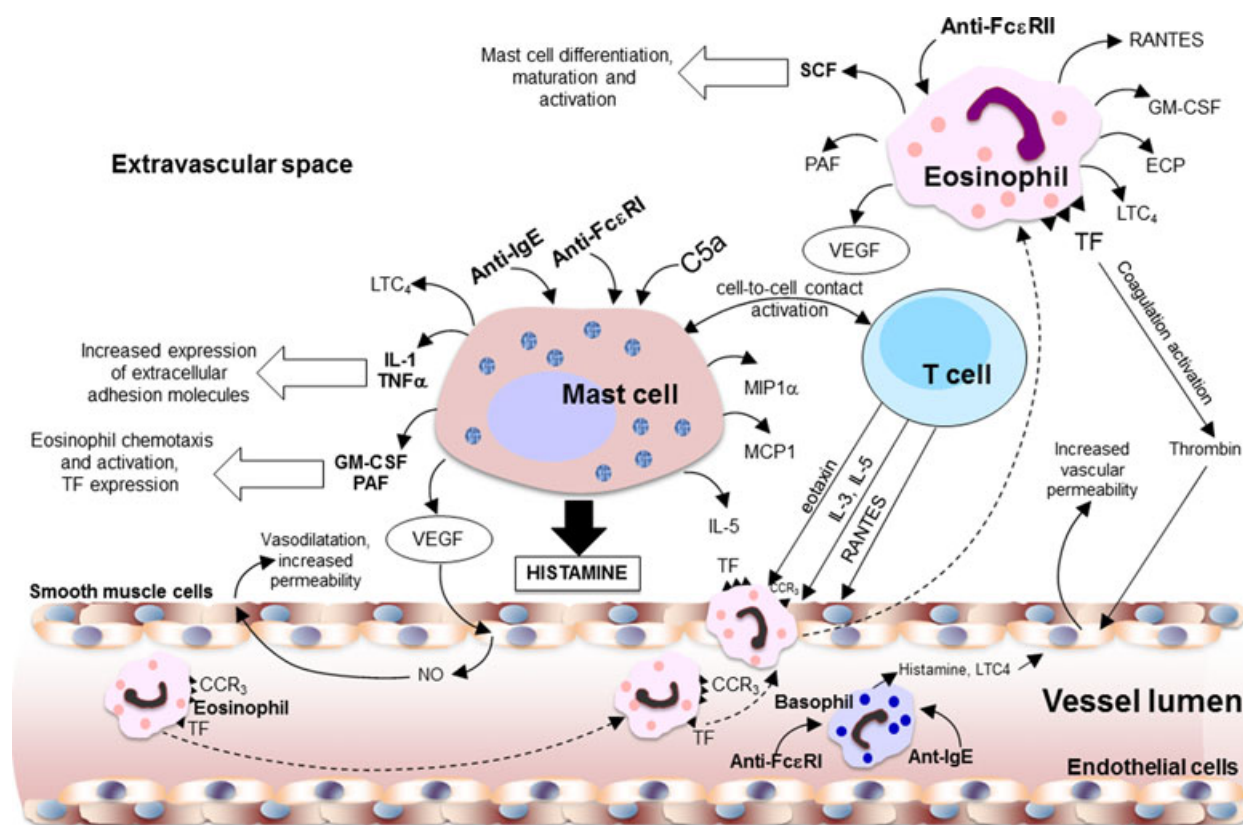


Figure 2 Summary of the events possibly involved in the pathogenesis of chronic urticaria. Functional autoantibodies to the high-affinity IgE receptor (FcεRI) or to IgE induce histamine release intravascularly from basophils (lower side of the picture) and extravascularly from mast cells (MCs) (upper side of the picture). Activated MCs and basophils release several inflammatory mediators, chemokines and cytokines. Contemporarily, eosinophils, activated by autoantibodies to the low-affinity IgE receptor (FcεRII) and/or by cytokines, express tissue factor and thus trigger the activation of the coagulation cascade. This leads to the generation of thrombin which causes vasodilation, increases vascular permeability and induces direct MC degranulation. In addition, eosinophils release a

number of inflammatory mediators including the major basic protein (MBP) which in turn can provoke MC degranulation. Activated T cells may participate through activation of MCs by cell-to-cell contact and by release of multifunctional cytokines and chemokines (31). CCR3: chemokine receptor 3; ECP: eosinophil cationic protein; GM-CSF: granulocyte macrophage colony stimulating factor; IL-1: interleukin-1; IL-3: interleukin-3; IL-5: interleukin-5; LTC4: leukotriene C4; MCP-1: monocyte chemotactic protein-1; MIP-1α: macrophage inflammatory protein; NO: nitric oxide; PAF: platelet-activating factor; RANTES: regulated on activation, normal T cell expressed and secreted; SCF: stem cell factor; TF: tissue factor; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor.

level, but not thrombin-antithrombin complex, correlated with disease activity. This suggests that FVIIa might play an important role in CU pathogenesis. Takeda et al. (40) observed an increase in coagulation potential in CU patients with the involvement of the intrinsic coagulation factors. In addition to that, thrombin can cause degranulation of MC via PAR-1 and may increase vascular permeability by a direct effect on endothelial cells (34, 35, 41). The available data support the role of eosinophils as a source of TF and subsequent activation of the coagulation cascade in CU pathogenesis (22, 38). Moreover, TF promotes transendothelial migration of eosinophils (Fig. 2). Accordingly, Moosbauer et al. (21) showed that specific antibodies to TF extracellular domain significantly inhibited the early phase of eosinophil migration through IL-4 activated endothelium. Finally, ample evidence exists on the close link between coagulation and inflammation (42). At the cellular level, the coagulant mediators act on PARs inducing the expression of proinflammatory cytokines (43). Proinflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF- α) induce the expression of TF (42). The two systems (coagulation and inflammation) activate each other, thus increasing the response and possibly contributing also to increase vascular permeability (10, 15).

Coagulation biomarkers in chronic urticaria

Patients with active CU often demonstrate changes in the levels of various coagulation biomarkers, particularly prothrombin fragment F1 + 2, activated factor VII (FVIIa) and D-dimer. F1 + 2 is a polypeptide of about 34 kD that is released into the circulation during the activation of prothrombin to thrombin by activated factor X (FXa; 44). FVIIa is generated following the activation of FVII by tissue factor. D-dimer is a fibrin degradation product of 180 kD released in blood after the lysis of thrombus (fibrinolysis; 45). Asero et al. (37) first observed elevated plasma levels of prothrombin fragment F1 + 2 which were related to urticaria severity, suggesting that the severity of the disease is paralleled by the amount of thrombin generated. Thrombin generation was demonstrated to be associated with the activation of the extrinsic (tissue factor) pathway because, in CU patients, increased plasma levels of activated factor VII (FVIIa) were observed, whereas no activation of factor XII was seen (38). These data are in line with the early observations by Bork et al. (46), who found a reduction of factor VII associated with an increase of factor XII in ten patients with CU, indicating an activation and subsequent consumption of factor VII but not of factor XII. Significantly elevated plasma levels of F1 + 2 and D-dimer were found in patients with severe exacerbation of CU, showing that in this subset of patients the coagulation cascade and fibrinolysis are activated. Interestingly, D-dimer plasma levels dropped to normal values during CU remission (47). The activation of blood coagulation in patients with CU has been confirmed by other researchers (39, 48–50). In particular, Wang et al. (39) found increased plasma levels of FVIIa (marker of the activation of the TF pathway), thrombin-antithrombin complex

(marker of thrombin generation) and D-dimer (marker of fibrin degradation), which are correlated to each other in CU patients. Thrombin-antithrombin complex and D-dimer plasma levels were also found increased in CU by Fujii et al. (48). Khalaf et al. (49) and Yildiz et al. (50) confirmed high levels of F1 + 2 during active CU and a significant decrease during remission.

Zhu et al. showed a statistically significant increase in F1 + 2 and thrombin-antithrombin complexes in CU patients (51). In another study, Takeda et al. noted a significant increase in plasma levels of fibrinogen, D-dimer, fibrin and fibrin degradation products, as well as the soluble fibrin monomer complexes in 36 patients with CU. All parameters correlated with CU severity, but the increase in the level of prothrombin fragment F1 + 2 was not statistically significant (40). In patients with CU, the increased level of D-dimer and its association with disease severity has been also confirmed in other studies (52–54). In addition, it has been shown that patients with active CU have elevated plasma D-dimer levels compared to patients with CU in remission and patients with psoriasis (53).

In CU, besides the coagulation, the vascular involvement is supported by the finding that patients had elevated plasma levels of VEGF (24), which is one of the most potent regulators of angiogenesis and a major mediator of vascular permeability. Vascular endothelial growth factor vasodilatory effect occurs via the production of nitric oxide (NO) by endothelial cells (55). It should be noted that eosinophils are an important source of VEGF in CU patients, which is consistent with observations *in vitro*, indicating the ability of peripheral blood eosinophils to induce angiogenesis (23, 25). Vascular endothelial growth factor level correlates with the severity of the disease and is associated with the level of F1 + 2 fragment in plasma and thus with the activation of the coagulation cascade (24).

Activation of coagulation cascade in other related diseases

Activation of coagulation has been demonstrated in patients with AE associated with C1-inhibitor deficiency; in fact increased levels of F1 + 2 and D-dimer were found during acute attacks of edema (16, 56). The deficiency of C1-inhibitor, the main inhibitor of the contact system, renders the components of this system, mainly factor XII, more prone to the activation, particularly after stimulations such as trauma or surgery. Contact system mediates pro-coagulation and proinflammatory reactions through the intrinsic coagulation pathway and kallikrein-kinin system, respectively (57). The activation of the contact system leads to the release of the nonapeptide bradykinin, a potent mediator increasing vascular permeability and thus causing AE. The subsequent activation of the coagulation cascade leads to thrombin generation which may contribute in the pathogenesis of edema. Hyperactivation of the contact system may occur also in a form of hereditary AE not associated with C1-inhibitor deficiency and characterized by a gain of function mutation in factor XII gene (58, 59).

Marzano et al. assessed the plasma levels of coagulation activation markers, such as F1 + 2 and D-dimer, in 63 patients with bullous pemphigoid (BP) and in patients with different skin diseases as CU ($n = 20$), atopic dermatitis ($n = 14$), skin reactions to drugs ($n = 6$), psoriasis ($n = 20$), dermatitis herpetiformis ($n = 4$), primary cutaneous T-cell lymphoma ($n = 5$) and in 40 healthy volunteers. BP is a skin disease characterized by the production of autoantibodies to hemidesmosomal BP180 and BP230 proteins and by eosinophil involvement in the formation of blisters. TF, the initiator of coagulation, was found in eosinophilic granules and was released upon activation. F1 + 2 and D-dimer levels were higher in the plasma of patients with BP, than in the control group, positively correlated with the severity of the disease, the eosinophil count and anti-BP180 antibodies. TF expression was observed only in patients with BP, CU and atopic dermatitis. The authors concluded that the activation of the coagulation cascade is present in patients with BP and other eosinophil-associated skin diseases, but is absent in noneosinophilic disorders (15).

Patients with urticarial vasculitis (52) and delayed pressure urticaria (60) had increased D-dimer levels. Kasperska-Zajac et al. measured the concentration of D-dimer in plasma from eight patients with delayed pressure urticaria with different severity. In four patients, plasma levels of D-dimer were higher than the upper reference range. These patients had severe urticaria and marked rash. In the other cases, the symptoms of urticaria were less severe, and the concentration of D-dimer was within normal limits. The authors suggested that patients with severe delayed pressure urticaria could develop hyperfibrinolysis, associated with increased D-dimer level, possibly due to a systemic inflammatory response (60). Similarly to patients with CU, patients with the multiple drug allergy syndrome had signs of thrombin generation in most cases, as demonstrated by the significant increase in F1 + 2 plasma levels (61).

The activation of the coagulation cascade was also noted in patients with nonallergic asthma. The average levels of F1 + 2 fragments, D-dimer and VEGF were significantly higher in patients with asthma than in the control group. These patients had the tendency to show a more severe disease according to GINA (Global Initiative for Asthma) classification. Interestingly, 19 of 21 patients (90%) had a positive autologous plasma skin test (APST; 62), suggesting the presence of circulating histamine-releasing factors and/or vasoactive substances related to coagulation activation and angiogenesis.

Autoantibodies, complement and coagulation

Substantial advances in our understanding of the pathogenesis of CU were recorded in 1986 with the observations by Grattan et al. (63) that the intradermal injection of autologous serum caused a wheal-and-flare reaction in a significant proportion of CU patients. Such a finding suggested the presence of circulating histamine-releasing factors as a possible pathogenic factor. A couple of years later, Gruber et al. (4) detected nonfunctional (i.e. not able to induce basophil

degranulation *in vitro*) IgG anti-IgE autoantibodies in patients with CU or cold urticaria. In 1991 Grattan et al. (64) found for the first time functional anti-IgE autoantibodies, and 2 years later the same group concluded that an IgG autoantibody specific for the α subunit of the high-affinity IgE receptor (Fc ϵ RI) was the main mediator of whealing in CU (65). Such autoantibody was detectable in about one-third of CU patients (66). This finding was confirmed by independent groups (67, 68), and a subsequent study in 78 CU patients found that functional anti-IgE antibodies can be detected in 9% of cases (69). The functional autoantibodies involved in the pathogenesis of CU belong to the IgG1 and IgG3 subclasses, which have been demonstrated to fix the complement system and induce histamine release (70–72). The release of histamine induced by anti-Fc ϵ RI autoantibodies has been demonstrated both from MCs (73) and basophils (65).

Experimental data showed that thrombin not only activates PAR-1 receptors on MCs but also increases vascular permeability by direct action on endothelial cells (74, 75) and can activate C5, bypassing the first stage of complement cascade and generating C5a in the absence of C3 (76). This relationship between the complement and coagulation may be a new way of complement activation. Recently, Zhu et al. (51) found increased levels of activated complement component 5 (C5a) in patients with active CU further supporting the involvement of complement system in the pathophysiology of the disease.

Chronic urticaria is a persisting inflammatory disorder, characterized by MC degranulation and perivascular, non-necrotizing infiltrate of lymphocytes, consisting of a mixture of Th1 and Th2 subtypes as well as monocytes, neutrophils, eosinophils and basophils (77, 78). In active CU, local cutaneous infiltrate is accompanied by a systemic inflammatory process indicated by the increased circulating C reactive protein (CRP), interleukin-6 (IL-6) and matrix metalloprotease 9 (MMP-9; 79, 80).

Ample evidence exists on the close link among the immune response, inflammation and coagulation (42, 81). Proinflammatory cytokines such as IL-6 and tumor necrosis factor alpha (TNF- α) induce the expression of tissue factor, the main initiator of blood coagulation (42). Tissue factor activates the coagulation cascade generating thrombin which leads to the formation of fibrin clot from fibrinogen. At the cellular level, the coagulant mediators act on protease-activated receptors (PARs) inducing the expression of proinflammatory cytokines (43). In turn, the two systems – coagulation and inflammation – activate each other, thus increasing the response. In 2006, Asero et al. (37) noted that intradermal injection of plasma instead of serum led to the significant increase in vascular permeability. The authors interpreted this observation as evidence for the possible role of thrombin in the pathogenesis of urticaria and of its ability to increase vascular permeability possibly by the activation of endothelial cells and MC (37). In a more recent study, the same authors have noted that the activation of the coagulation cascade was more pronounced in patients with CU and positive ASST than in patients with negative results (82).

Increased F1 + 2 prothrombin fragments and D-dimer levels were observed in seven of 16 APST-negative CU patients, most of whom were males (10/6). Activation of the coagulation cascade was associated with severe CU. Mean levels of F1 + 2 and D-dimer in APST-negative patients were higher than those in the control group, but lower than in patients with a positive APST and autoreactive urticaria (82). Thus, ASST and APST results may depend not only on the presence of circulating antibodies to Fc ϵ RI or IgE but also by other histamine-releasing factors or soluble mediators (37, 83).

Another interesting observation on the interaction between hemostasis and inflammation comes from the study of Magen et al. who found increased mean platelet volume (MPV) and C-reactive protein in ASST-positive CU patients showing a more severe course of the disease. The alteration in platelet size might indicate a direct role for activated platelet mediators in urticaria or simply reflect bone marrow stimulation induced by increased systemic inflammation (84).

Discussion

Several lines of evidence suggest that coagulation, fibrinolysis and complement systems have a role in CU pathogenesis and that the evaluation of their biomarkers may be of help in the disease management. In particular, the level of D-dimer in plasma correlates with disease severity, and, although studies have been carried out only on few patients, it has been found that it returns to normal values during remission. Despite its relative nonspecificity (as it may be elevated in a number of inflammatory processes), measurement of D-dimer can be proposed for assessing the severity of disease and possibly predicting the response to treatment with antihistamines in patients with CU (47, 85, 86).

Hypercoagulation in patients with urticaria may contribute to inflammation and tissue damage, and theoretically might also increase the risk of thrombosis. Whether activation of coagulation is a primary phenomenon involved in the CU pathophysiology or a secondary process, enhancing or maintaining urticarial inflammation is a matter of debate (7). However, it is likely that coagulation participates to CU pathophysiology even when the main pathogenetic mechanism relies on histamine-releasing autoantibodies. It has been suggested that patients with long-term CU, accompanied by severe systemic inflammation, particularly with high blood levels of CRP, may have an increased risk of cardiovascular disorders (87). Other observations indicated that the hypercoagulation occurring in CU and AE is unlikely to be associated with an increased risk of thrombosis, in contrast to patients with acute urticaria (11). So far, an increased incidence of thrombotic events in patients with long-lasting CU has not been documented. Possible explanations for the lack of an increased thrombotic risk, in spite of hypercoagulation, are an efficient activation of fibrinolysis and anticoagulation system as well as the predominant extravascular location of fibrin deposition. In fact Fujii and co-workers (48) found that the coagulation biomarkers that

we routinely measure in patients with CU reflect an activation of the coagulation cascade that in CU occurs extravascularly, which may explain the low prevalence of thrombosis in this disease. In contrast, the activation of coagulation in patients with other skin diseases such as BP is associated with an increased thrombotic risk (9, 88). Chronic urticaria is frequently characterized by the presence of elevated levels of plasma markers of thrombin generation and fibrinolysis during severe exacerbations of the disease (37, 38, 47), which can be due to the expression of TF by activated eosinophils (22). The activation of coagulation and fibrinolysis decreases till complete normalization during remission (47, 89). Whether the activation of coagulation/fibrinolysis has a main role in the pathogenesis of the disease or simply acts as an amplification system has still to be defined. However, the fact that such an activation parallels the activity of CU may provide the rationale for an anticoagulant and antifibrinolytic therapy in patients with severe CU. The effectiveness of anticoagulant therapy in some patients with refractory CU was observed more than a decade ago (90) by the use of oral anticoagulants and more recently by the use of heparin (14, 91, 92). The use of heparin can be effective in patients with urticaria who have high levels of D-dimer and poor response to antihistamines (14, 85). A significant decrease in disease symptoms was observed by Asero et al. (14) in five of eight patients with CU when nadroparin (11 400 IU per day) and tranexamic acid (per os 1 g three times a day for 2 weeks) were added to H1-antihistamines. The remission of CU was also reported by Chua et al. (91) in a 43-year-old woman on heparin therapy who had been previously treated with antihistamines without any effect. Finally, Spring et al. (92) reported a 59-year-old woman who had been suffering from CU for 7 years and was successfully treated first with nadroparin and then with acenocoumarol because of an intercurrent deep vein thrombosis experiencing the complete disappearance of CU symptoms.

An interesting, indirect confirmation of the possible role of the coagulation pathway in the pathogenesis of CU comes from recent studies reporting the efficacy of serine protease inhibitors nafamostat mesilate and camostat mesilate in refractory CU (93). Such drugs inhibit different proteases, including tryptase, kallikrein, complement, factor XII, and plasmin, and show an anticoagulant effect similar to that of heparin (94).

Finally, the fact that functional anti-Fc ϵ RI and anti-IgE autoantibodies do not explain all cases of CU, is further supported by the study of Bossi et al. (95), who found that CU sera can induce degranulation of MCs which do not express the high-affinity IgE receptor. This finding opens up additional opportunities for understanding the pathogenesis of CU and discovering new histamine-releasing factors, especially in patients without increased coagulation markers, autoreactivity and circulating autoantibodies.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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