Acta Dermatovenerologica Alpina, Pannonica et Adriatica **2024; doi: 10.15570/actaapa.2024.13**

Original article

Analysis of coagulation factors in angioedema/urticaria: increased values of D-dimer and fibrinogen in isolated angioedema

Maja Štrajtenberger,[1] Liborija Lugović-Mihić,[2,3]✉ Asja Stipić-Marković,[1] Marinko Artuković,[1,4] Roman Mihić,[5] Lorena Dolački,[6] Nika-Barbara Pravica,[7] Ivica Lokner[1,8]

[1] *Department of Pulmonology, Special Hospital for Pulmonary Diseases, Zagreb, Croatia*
[2] *Department of Dermatology, University Hospital Center Sestre Milosrdnice, Zagreb, Croatia*
[3] *School of Dental Medicine, University of Zagreb, Zagreb, Croatia*
[4] *Osijek Faculty of Dental Medicine and Health, Osijek, Croatia*
[5] *Department of Clinical Chemistry, University Hospital Center Sestre Milosrdnice, Zagreb, Croatia*
[6] *Institute of Emergency Medicine of Zagreb County, Velika Gorica, Croatia*
[7] *Department of Emergency Medicine, University Hospital Center Sestre Milosrdnice, Zagreb, Croatia*
[8] *Department of Laboratory Diagnostics, Department of Pulmonology, Special Hospital for Pulmonary Diseases, Zagreb, Croatia*

Abstract

Introduction: Recent research has shown that blood coagulation and the extrinsic coagulation cascade are involved in the pathogenesis of chronic spontaneous urticaria (CSU), but little is known about the coagulation factors in angioedema.
Methods: This study included 58 participants: 29 patients with chronic angioedema (14 with isolated angioedema and 15 with angioedema with wheals) and 29 healthy controls (HCs). We compared the values of coagulation factors in patients with isolated angioedema to those with wheals. Plasma levels of D-dimer, fibrinogen, and factor VII were measured by enzyme-linked immunosorbent assay (ELISA) for all participants.
Results: Significantly higher D-dimer ($p = 0.016$; $\varepsilon^2 = 0.381$) and fibrinogen ($p = 0.044$; $\varepsilon^2 = 0.331$) levels were recorded in patients with angioedema (both groups) than in the HCs, with higher levels for angioedema with wheals. Factor VII and fibrinogen levels did not differ significantly between the groups with angioedema, but coagulation factors were more often elevated in both angioedema groups than in HCs.
Conclusions: One characteristic of angioedema is an elevated blood coagulation potential, which may help produce fibrin and may be important in controlling angioedema attacks.

Keywords: angioedema, urticaria, D-dimer, fibrinogen, factor VII

Received: 30 January 2024 | **Returned for modification**: 10 March 2024 | **Accepted**: 17 April 2024

Introduction

Angioedema is a painless and limited swelling of the subcutaneous or submucosal tissue, typically occurring in episodes affecting areas such as the face, lips, oral cavity, genitalia, or gastrointestinal regions (1–9). Attacks are unpredictable and asymmetrical. Angioedema can occur in isolation or in association with wheals (urticaria) in approximately 40% to 60% of patients (4–7). By definition, urticaria is a condition characterized by the development of wheals (hives), angioedema, or both (1, 2). Based on its duration, urticaria is classified as acute or chronic, and according to definite triggers it is classified as inducible or spontaneous (1). Chronic

✉ liborija@sfzg.hr

spontaneous urticaria (CSU) is defined by the presence of wheals, angioedema, or both for more than 6 weeks and can be characterized by angioedema, excruciatingly itchy recurrent hives, or both.

Angioedema can be seen in mast cell–mediated disorders, bradykinin-mediated disorders, or other conditions (infections and some rare disorders), or it can appear as idiopathic angioedema (8). Angioedema is the result of fluid extravasation from increased vascular permeability, which is mediated by vasoactive mediators. There are two main types of angioedema: the rare hereditary angioedema (HAE) and the more common non-hereditary angioedema (non-HAE) (9–11). Angioedema is also classified by mediators, the two key mediators involved in its pathogenesis being histamine and bradykinin. The variant mediated by bradykinin can be hereditary or acquired; for example, angioedema caused by angiotensin-converting enzyme (ACE) inhibitor. Angioedema mediated by histamine is mostly an allergic type, but it can also be caused by direct mast cell / basophil activation and consequent release of inflammatory mediators or by disruption of the arachidonic acid pathway (9–19). The third type has a combination of mediators (e.g., angioedema caused by pseudoallergic reactions to non-steroidal anti-inflammatory drugs). There has been a recent increase in the incidence of all types of angioedema, including drug-mediated angioedema, such as those triggered by ACE inhibitors or vaccines, and allergic angioedema, which is in line with the general increase in prevalence of allergic diseases (7, 12, 19). Although there are clear categories of angioedema, the mechanisms responsible for the differences between isolated angioedema and angioedema with wheals remain to be fully elucidated.

In diagnostics, the causes of angioedema are established based on clinical symptoms (including related autoimmune or infectious diseases), deficiency of C1 inhibitor (C1-INH), and factor XII mutation. Because there is no standard defined methodology for diagnosing angioedema, the recommendations for diagnosing and treating CSU should be followed. The blood tests for these patients include a differential blood count (DBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), total IgE antibodies, and anti-thyroid peroxidase (TPO) IgG antibodies, among others (1, 2, 20–22). Bloodwork shows that both isolated and angioedema with wheals are associated with higher inflammatory markers (CRP, ESR), and serum values for D-dimers and fibrinogen are often used as additional biomarkers of angioedema severity (21–25). Concerning therapy, histaminergic angioedema is treated with antihistamines, corticosteroids, and epinephrine, or even anti-IgE receptor omalizumab (for idiopathic angioedema). Treatment for non-histamine-mediated angioedema involves blocking bradykinin release or activity. No licensed treatments for the remaining types of isolated angioedema exist (1, 26–28).

According to research data on chronic urticaria, the blood coagulation cascade plays an active role in the disease; specifically, inflammation and coagulation activate each other (21, 22, 25, 29–35). In both urticaria and angioedema, the activation of skin mast cells takes place through the external coagulation cascade and can be explained by a combination of infection (e.g., Gram-negative bacteria) and histamine and other tissue factor (TF) inducers including tumor necrosis factor alpha (TNF-α), interleukins (IL)-1β and IL-33, and *v*ascular endothelial growth factor (VEGF), leading to changes in vascular endothelial cells. Thus, CSU pathogenesis involves the extrinsic coagulation cascade triggered by TF and complement factors (such as C3a and C5a), although it is unclear how the TF-triggered coagulation pathway and complement factors induce the activation of skin mast cells and peripheral basophils in CSU patients (29–31). It seems that TF, expressed on vascular endothelial cells, eosinophils, or monocytes, then activates the extrinsic coagulation cascade, which leads to the production of the active forms of coagulation factors, such as factor (F) VIIa, FXa, and FIIa. Thus, FXa and FIIa increase vascular permeability via protease-activated receptor 1 (PAR-1), expressed on vascular endothelial cells, and induce leakage of plasma containing active coagulation factor forms and complement components. Active coagulation factor forms, such as FXa and FIIa, may convert serum C5 to C5a in intravascular and leaked plasma, which then induces mast cells / basophils to release histamine via C5aR. In addition, a fibrinolysis factor, plasmin, converts C5 to C5a, resulting in mast cell activation (29). In response to C5a and/or autoantibodies, the activated dermal mast cells / basophils release a large amount of histamine, which results in long-lasting formation of edema and wheals, through H1 receptors expressed on vascular endothelial cells. Meanwhile, activated eosinophils migrate outside of blood vessels and release stored inflammatory mediators, which may also induce or enhance the degranulation of skin mast cells via Mas-related G protein-coupled receptor X2 (MrgX2). However, in patients with CSU (possibly also angioedema), elevated levels of several molecules (including coagulation factors) have been found: D-dimers,

fibrin, prothrombin fragments 1 and 2, CRP, proinflammatory cytokines TNF-α, transforming growth factor-β (TGF-β), and IL-1, IL-6, IL-17, IL-31, and IL-33 (20–22, 35). So far, there is no evidence of elevated levels of these molecules in isolated recurrent angioedema Thus, because recent research has indicated the involvement of the extrinsic coagulation cascade in CSU pathogenesis, and because little is known about the coagulation pathway's connection with angioedema, we wanted to examine and compare the values of key coagulation factors in patients with isolated angioedema and patients with angioedema with wheals.

Methods

Participants and collection of blood samples

This study was conducted in accordance with the Declaration of Helsinki, and it was approved by the Ethics Committee of University Hospital Center Sestre Milosrdnice in Zagreb, Croatia, in December 2022 (protocol no. 251-29-11-21-08) for studies involving humans.

Participants were gathered at the Department of Immunology and Rheumatology at the Special Hospital for Pulmonary Diseases in Zagreb. We collected patients with chronic angioedema based on the criteria for chronic urticaria, which is defined as the occurrence of wheals, angioedema, or both for more than 6 weeks (1). We excluded patients with hereditary angioedema, acute urticaria, inducible (physical and non-physical) urticaria, or any form of urticaria other than spontaneous urticaria (1, 9). With adherence to these inclusion and exclusion criteria, our study encompassed 29 patients diagnosed with chronic angioedema, spanning both those with isolated angioedema and those with chronic angioedema accompanied by wheals. In total, our participant pool comprised three distinct groups: 14 patients with isolated angioedema, 15 patients with chronic angioedema accompanied by wheals, and 29 healthy controls (HCs). Therefore, the cumulative sample size amounted to 58 participants.

Coagulation assays

First, participants' blood was drawn into evacuated anticoagulant tubes, nine volumes of blood to one volume of 3.8% (weight/volume) trisodium citrate solution. After centrifugation for 15 minutes at 1,500 *g*, the platelet-poor plasma was stored at −80 °C and thawed at 37 °C immediately prior to performing the assays presented in Table 1 (factor VII, fibrinogen, and D-dimer).

D-dimer was measured on the Cobas Integra 400 plus analyzer (Roche Diagnostics, Vienna, Austria) using original manufacturer reagents and protocols. It was determined by latex-immunoturbidimetry using the original D-dimer reagent cassette D-DI2 and the D-dimer gen2 Calibrator Set. This method has been standardized against the Asserachrom D-dimer method. Factor VII and fibrinogen were measured on the BCS XP analyzer (Siemens Healthineers, Marburg, Germany) using the manufacturer's reagents and protocols. Fibrinogen concentration was determined by the Clauss clotting method with the Multifibren U reagent (Siemens Healthineers, Marburg, Germany). Finally, the activity of factor VII was measured with the coagulometric assay using factor VII–deficient plasma (Siemens Healthineers, Marburg, Germany). A mixture of factor VII–deficient plasma and patient plasma were tested with the Thromborel S (Siemens Healthineers, Marburg, Germany), and the results were interpreted as a percentage according to a calibration curve made by using dilutions of Standard Human Plasma (Siemens Healthineers, Marburg, Germany). Standard Human Plasma is traceable to the International Standard WHO 09/172.

Statistical analysis

Age and coagulation factor values were compared across groups using the Kruskal–Wallis test and post-hoc Mann–Whitney test with Bonferroni adjustment for multiple comparisons. Effect size was calculated with the formula $\varepsilon^2 = H / [(n^2 - 1) / (n + 1)]$ for the Kruskal–Wallis test, and $r = z / \sqrt{N}$ for the Mann–Whitney test. Cohen's criteria were used for interpretation—for the Mann–Whitney test, a small effect size (r) was 0.25–0.30, 0.30–0.50 was moderate, 0.50–0.70 was large, and > 0.70 was a very large effect size. The squared values of Cohen's criterion were used for the interpretation of ε^2 in the Kruskal–Wallis test. To compare frequency, the χ^2 test was utilized, and Cramer's V was used to calculate the effect's size. The aforementioned Cohen's criteria were applied as limiters for the assessment of the effect size. Spearman's correlation and

Cohen's interpretational standards were used to analyze relationships between variables. Two-way analysis of covariance was used to analyze whether there was an interaction between group and sex with the control of age at the level of coagulation factors, and the effect size was quantified using η^2 and interpreted with the help of the squared values of Cohen's criteria.

Results

Our study involved 58 participants, comprising individuals diagnosed with chronic angioedema/urticaria and HCs. The age range of the participants was from 21 to 79 years, with a median age of 45 years. The interquartile range was from 35.8 to 58.3 years. Among the participants, 79% were female (Table 1). The groups differed by age with a large effect size ($p = 0.001$; $\varepsilon^2 = 0.532$). The angioedema group was significantly older than HCs, with a large effect size ($p = 0.001$; $r = -0.611$; Fig. 1). Women were significantly younger than men, with a moderate effect size (median 42 vs. 59 years; $p = 0.017$; $r = -0.314$). There was also no significant difference in sex distribution between the groups (Fig. 2).

Based on the results for coagulation factor levels for the three groups, the groups differed in fibrinogen levels, with a moderate effect size ($p = 0.044$; $\varepsilon^2 = 0.331$), and D-dimer ($p = 0.016$; $\varepsilon^2 = 0.381$), but not in factor VII levels (Figs. 1 and 3). When comparing parameter levels, differences between the three groups were significant only for D-dimer, for which patients with angioedema with wheals had higher D-dimer levels than HCs, with a moderate effect size ($p = 0.004$; $r = -0.430$). There were no statistically significant differences in the proportions of participants with elevated coagulation factors; nonetheless, coagulation factors were more often elevated in patients with isolated angioedema and those with angioedema with wheals than in HCs (Table 2).

Our results also show that coagulation factor values correlated with age, and that these correlations were weak to moderate, linear, and positive. With increasing age, factor VII increased ($r = 0.270$; $p = 0.041$), as did D-dimer levels ($r = 0.308$; $p = 0.019$). Fibrinogen levels were primarily dependent on age ($r = 0.392$; $p = 0.002$).

Two-way analysis of covariance revealed that, when controlling for age, there was no interaction between sex and study groups regarding the levels of coagulation factors. Furthermore, D-dimer values mostly depended on the subject's group affiliation ($p = 0.035$; $\eta^2 = 0.123$), whereas fibrinogen levels mainly depended on age ($p = 0.009$; $\eta^2 = 0.126$). Additional statistics indicate that, when controlling data for age, sex, and data on specific angioedema groups, D-dimer primarily depends on the specific angioedema groups rather than age.

There were no significant differences in the levels of coagulation factors or the prevalence of elevated coagulation factors between men and women. Two-way analysis of covariance showed that, when controlling for age, there was no interaction between sex and study groups on the level of coagulation factors. Moreover, D-dimer values mostly depended on the subject's group affiliation ($p = 0.035$; $\eta^2 = 0.123$), and fibrinogen mainly depended on age ($p = 0.009$; $\eta^2 = 0.126$).

Discussion

This study is the first attempt to analyze values of coagulation factors in patients with isolated angioedema and compare them to values of coagulation factors in patients with angioedema with wheals and HCs. By conducting coagulation assays for the levels of coagulation factors and markers of activated coagulations, we were able to show that greater coagulation potential is also highly related to isolated angioedema, not only to angioedema accompanied with wheals. Using traditional coagulation assays, we assessed hemostatic characteristics, for which both patients with isolated angioedema and patients with angioedema with wheals had greater levels of fibrinogen and D-dimer than HCs. The increase of factor VII levels, however, was not significant when compared with HCs. Although we did not find statistically significant differences in the proportions of participants with elevated coagulation factors, coagulation factors were more often elevated in patients with chronic angioedema (isolated angioedema and angioedema with wheals) than in HCs. It indicates that one of the characteristics of angioedema is an elevated blood coagulation potential, which may help

produce fibrin and may be important in controlling angioedema attacks.

The etiology of newly formed isolated angioedema is challenging to differentiate, which may be important, particularly in emergency or clinical practice (7). Given that the angioedema group encompasses three main subgroups (histamine-mediated, bradykinin-mediated, and idiopathic angioedema types), the absence of a biological diagnostic marker for these angioedemas is why bradykinin-mediated angioedema is sometimes treated with antiallergic therapy and why clinicians rarely administer fast-acting bradykinin antagonists. However, over the last decade, significant progress has been made in understanding the pathophysiology of angioedema, especially HAE. Thus, in addition to the gene for C1-INH, several new mutations for proteins involved in blood enzyme cascades have been discovered (FXII, plasminogen, and angiopoietin) (7). Consequently, the deficiency of these particular enzymes disrupts the balance between blood enzymatic systems, such as the kinin–kallikrein system, the renin–angiotensin system, the contact coagulation pathway, fibrinolysis, and the complement pathway. This disruption leads to an excess of bradykinin formation and increased vascular permeability. On the other hand, the development of histamine-induced angioedema differs, arising from an immune reaction (whether allergic or autoimmune), resulting in histamine release, vasodilation, and increased vascular permeability. The role of coagulation in the pathogenesis of chronic urticaria, with or without angioedema, has been known for a long time. In addition, in HAE (bradykinin-mediated), there is clinical experience supporting the beneficial effect of coagulation blockage by tranexamic acid. However, it remains unclear how coagulation factors activate skin mast cell and peripheral basophils in chronic angioedema or chronic urticaria. Furthermore, the mechanisms responsible for isolated angioedema versus chronic angioedema are not yet clearly understood.

In addition, although the pathogenesis of chronic urticaria and angioedema has not been fully elucidated, according to current knowledge, the autoantibodies IgE and IgG predispose mast cell / basophil activation, with the involvement of coagulation and complement cascades. In their pathogenesis, the activation of skin mast cells takes place through the external coagulation cascade and may be associated with various microorganisms, cytokines, immune factors, and other blood factors (6, 30, 36–40). Concerning autoimmunity in CSU, according to several reports, around 30% to 50% of CSU patients have IgG autoantibodies against IgE or the high-affinity IgE receptors (FcεRIs) expressed on mast cells / basophils, which induce this cell activation, and subsequent release of stored mediators. Moreover, in certain CSU patients, IgE autoantibodies against several molecules such as double stranded DNA, TF, IL-24, and TPO have been detected. A role of IgG autoantibodies against IgE or IgE autoantibodies against self-molecules in CSU has been supported by the effectiveness of omalizumab (anti-IgE monoclonal antibody) in these patients.

According to previous studies, the severity of angioedema with wheals positively correlated with D-dimer levels in plasma (which may indicate this possibility in isolated angioedema as well) (24, 25). Elevated plasma levels of D-dimer and fibrinogen in patients with urticaria/angioedema may suggest an activation of the coagulation pathway and fibrinolysis. Activation of the coagulation pathway leads to thrombus formation, followed by activation of anticoagulation and fibrinolysis. The interaction between inflammation and coagulation creates a loop that maintains and enhances both systems (41). Generally, in our study, D-dimers were significantly increased only in patients with angioedema with wheals, with levels higher than those observed in HCs. The difference was not significant for patients with isolated angioedema. It is also worth noting that D-dimer values are elevated in several patients without thrombosis (this assay has a negative predictive value to exclude thrombosis) and that an increase in D-dimer is observed in patients with extravascular fibrin deposition. In patients with angioedema, the increase in D-dimer may be related, at least in part, to the increased vascular permeability and plasma extravasation into the surrounding tissues (7).

In addition to autoimmune/immune-mediated cutaneous illnesses, several inflammatory conditions also exhibit high levels of coagulation and fibrinolysis activation indicators. The activation of coagulation may have two major effects: a) it may play a local pathogenic role in causing skin lesions, and b) it may play a systemic role in raising the risk of thrombosis. Moreover, eosinophils express tissue factor in the skin microenvironment of chronic urticaria/angioedema patients, which can immediately stimulate coagulation by producing mediators of vascular permeability. Regarding the pathophysiology of skin diseases, thrombin helps increase endothelial vascular permeability, which amplifies the inflammatory network in chronic angioedema and chronic urticaria.

Concerning the association between urticaria and vascular factors (and related potential therapy based on this association), several reports were found of cardiovascular incidents (e.g., myocardial infarction) occurring during episodes of acute urticaria (42–45). This raises the question of whether anticoagulant medication may be indicated, especially when additional cardiovascular risk factors are present. This hypothesis is supported by the effectiveness of numerous anticoagulants, including warfarin, heparin, and nafamostat mesylate, which have been used in a few cases with chronic urticaria (46–50). According to Meyer-De Schmid and Neuman, the treatment of four chronic urticaria patients receiving intravenous unfractionated heparin leads to persistent remission (51). Furthermore, when subcutaneous unfractionated heparin was administered to a chronic urticaria patient resistant to antihistamine and immunosuppressive treatment, Chua and Gibbs described complete remission, which was followed by a relapse when heparin was stopped (48). For warfarin, an oral anticoagulant, reduced clinical symptoms were seen in six out of eight patients with antihistamine-resistant chronic urticaria in a small double-blind, placebo-controlled experiment (52). In an uncontrolled study, four out of five chronic urticaria patients were able to achieve remission with warfarin at a therapeutic dose (53). According to Asero et al., patients with severe chronic urticaria that are resistant to antihistamines and have high levels of D-dimer may benefit from a combination of low-molecular-weight heparin and tranexamic acid therapy, which is successful in treating patients with idiopathic angioedema or angioedema brought on by a lack of C1-inhibitor (49). All of these data, when considered collectively, point to the relevance of coagulation cascade activation in chronic angioedema and chronic urticaria and support the use of anticoagulant and antifibrinolytic medications for both diseases, particularly in the presence of additional cardiovascular risk factors.

Although it is novel, a limitation of this research is the small number of participants, a restricted number of examined coagulation parameters, and age differences among the three groups. Thus, the healthy subjects were significantly younger than the patients with isolated angioedema and the patients with angioedema with wheals, which may affect the results obtained. Therefore, further research, encompassing a larger number of parameters examined, is needed.

Conclusions

According to our research, significantly higher D-dimer and fibrinogen levels were recorded in patients with chronic angioedema (including those with isolated angioedema and those with angioedema with wheals) than in the HCs, with higher levels in those with angioedema with wheals. In addition, elevated coagulation factors were recorded more often in both angioedema groups than in HCs. This indicates that one of the characteristics of angioedema is an elevated blood coagulation potential, which may help produce fibrin and may be important in controlling angioedema attacks. This insight may potentially be important for controlling angioedema attacks, contributing to both general knowledge about angioedema and the pathogenetic network of angioedema. In turn, this greater knowledge will help researchers and clinicians explore new possibilities for therapy. More clinical experience and further research with a greater number of participants are needed for a fuller picture of the relationship between coagulation factors and the pathogenesis of angioedema.

References

1. Zuberbier T, Abdul Latiff AH, Abuzakouk M, Aquilina S, Asero R, Baker D, et al. The international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. Allergy. 2022;77:734–66.
2. Zuberbier T, Bernstein JA, Maurer M. Chronic spontaneous urticaria guidelines: what is new? J Allergy Clin Immunol. 2022;150:1249–55.
3. Azmy V, Brooks JP, Hsu FI. Clinical presentation of hereditary angioedema. Allergy Asthma Proc. 2020;41:S18–21.
4. Pozderac I, Lugović-Mihić L, Artuković M, Stipić-Marković A, Kuna M, Ferček I. Chronic inducible urticaria: classification and prominent features of physical and non-physical types. Acta Dermatovenerol Alp Pannonica Adriat. 2020;29:1–8.

5. Weller K, Maurer M, Bauer A, Wedi B, Wagner N, Schliemann S, et al. Epidemiology, comorbidities, and healthcare utilization of patients with chronic urticaria in Germany. J Eur Acad Dermatol Venereol. 2022;36:91–9.
6. Zhou B, Li J, Liu R, Zhu L, Peng C. The role of crosstalk of immune cells in pathogenesis of chronic spontaneous urticaria. Front Immunol. 2022;13:879754.
7. Stipić-Marković A. Nasljedni (hereditarni) angioedem i dijagnostičko-terapijske mogućnosti njegova ranog prepoznavanja i učinkovitog liječenja. In: Lugović-Mihić L, editor. Urtikarije i egzemi/dermatitisi. Zagreb: Medicinska naklada; 2022. p. 27–45. Croatian.
8. Nedelea I, Deleanu D. Isolated angioedema: an overview of clinical features and etiology. Exp Ther Med. 2019;17:1068–72.
9. Bellanti JA, Settipane RA. Hereditary angioedema again revisited. Allergy Asthma Proc. 2021;42:105–7.
10. Porebski G, Kwitniewski M, Reshef A. Biomarkers in hereditary angioedema. Clin Rev Allergy Immunol. 2021;60:404–15.
11. Zingale LC, Beltrami L, Zanichelli A, Maggioni L, Pappalardo E, Cicardi B, et al. Angioedema without urticaria: a large clinical survey. CMAJ. 2006;175:1065–70.
12. Stipić Marković A, Janžeković M. Increasing incidence of angioedema without urticaria—clinical features. Acta Med Croat. 2011;65:119–27.
13. Hahn J, Greve J, Bas M, Kojda G. Bradykinin-mediated angioedema induced by commonly used cardiovascular drugs. Drugs Drug Candidates. 2023;2:708–27.
14. Smolinska S, Antolín-Amérigo D, Popescu FD. Bradykinin metabolism and drug-induced angioedema. Int J Mol Sci. 2023;24:11649.
15. Napolitano F, Montuori N. The role of the plasminogen activation system in angioedema: novel insights on the pathogenesis. J Clin Med. 2021;10:518.
16. Wada A, Sawada Y, Sugino H, Nakamura M. Angioedema and fatty acids. Int J Mol Sci. 2021;22:9000.
17. Schettini N, Corazza M, Schenetti C, Pacetti L, Borghi A. Urticaria: a narrative overview of differential diagnosis. Biomedicines. 2023;11:1096.
18. Budimir J, Mravak-Stipetić M, Bulat V, Ferček I, Japundžić I, Lugović-Mihić L. Allergic reactions in oral and perioral diseases—what do allergy skin test results show? Oral Surg Oral Med Oral Pathol Oral Radiol. 2019;127:40–8.
19. Kostis WJ, Shetty M, Chowdhury YS, Kostis JB. ACE inhibitor-induced angioedema: a review. Curr Hypertens Rep. 2018;20:55.
20. Kuna M, Štefanović M, Ladika Davidović B, Mandušić N, Birkić Belanović I, Lugović-Mihić L. Chronic urticaria biomarkers IL-6, ESR and CRP in correlation with disease severity and patient quality of life—a pilot study. Biomedicines. 2023;11:2232.
21. Kolkhir P, André F, Church MK, Maurer M, Metz M. Potential blood biomarkers in chronic spontaneous urticaria. Clin Exp Allergy. 2017;47:19–36.
22. Kolkhir P, Pogorelov D, Olisova O. CRP, D-dimer, fibrinogen and ESR as predictive markers of response to standard doses of levocetirizine in patients with chronic spontaneous urticaria. Eur Ann Allergy Clin Immunol. 2017;49:189–92.
23. Baek YS, Jeon J, Kim JH, Oh CH. Severity of acute and chronic urticaria correlates with D-dimer level, but not C-reactive protein or total IgE. Clin Exp Dermatol. 2014;39:795–800.
24. Obtułowicz A, Migacz-Gruszka K, Pirowska M, Basta-Klonowska K, Wojas-Pelc A. Participation of the coagulation system and fibrinolysis as well as selected biomarkers in pathogenesis of chronic urticaria with various activity degree. Postepy Dermatol Alergol. 2020;37:608–12.
25. Asero R. D-dimer: a biomarker for antihistamine-resistant chronic urticaria. J Allergy Clin Immunol. 2013;132:983–6.
26. von Websky A, Reich K, Steinkraus V, Breuer K. Complete remission of severe chronic recurrent angioedema of unknown cause with omalizumab. J Dtsch Dermatol Ges. 2013;11:677–8.
27. Sands MF, Blume JW, Schwartz SA. Successful treatment of 3 patients with recurrent idiopathic angioedema with omalizumab. J Allergy Clin Immunol. 2007;120:979–81.

28. Wu MA, Zanichelli A, Mansi M, Cicardi M. Current treatment options for hereditary angioedema due to C1 inhibitor deficiency. Expert Opin Pharmacother. 2016;17:27–40.
29. Yanase Y, Takahagi S, Hide M. Chronic spontaneous urticaria and the extrinsic coagulation system. Allergol Int. 2018;67:191–4.
30. Yanase Y, Takahagi S, Ozawa K, Hide M. The role of coagulation and complement factors for mast cell activation in the pathogenesis of chronic spontaneous urticaria. Cells. 2021;10:1759.
31. Yanase Y, Matsubara D, Takahagi S, Tanaka A, Ozawa K, Hide M. Basophil characteristics as a marker of the pathogenesis of chronic spontaneous urticaria in relation to the coagulation and complement systems. Int J Mol Sci. 2023;24:10320.
32. Wang D, Tang H, Shen Y, Wang F, Lin J, Xu J. Activation of the blood coagulation system in patients with chronic spontaneous urticaria. Clin Lab. 2015;61:1283–8.
33. Cugno M, Asero R, Tedeschi A, Lazzari R, Marzano AV. Inflammation and coagulation in urticaria and angioedema. Curr Vasc Pharmacol. 2012;10:653–8.
34. Confino-Cohen R, Chodick G, Shalev V, Leshno M, Kimhi O, Goldberg A. Chronic urticaria and autoimmunity: associations found in a large population study. J Allergy Clin Immunol. 2012;129:1307–13.
35. Zhu H, Liang B, Li R, Li J, Lin L, Ma S, et al. Activation of coagulation, anti-coagulation, fibrinolysis and the complement system in patients with urticaria. Asian Pac J Allergy Immunol. 2013;31:43–50.
36. Krišto M, Lugović-Mihić L, Muñoz M, Rupnik M, Mahnic A, Ozretić P, et al. Gut microbiome composition in patients with chronic urticaria: a review of current evidence and data. Life. 2023;13:152.
37. Lugović-Mihić L, Bukvić I, Bulat V, Japundžić I. Factors contributing to chronic urticaria/angioedema and nummular eczema resolution—which findings are crucial? Acta Clin Croat. 2019;58:595–603.
38. Kocatürk E, Muñoz M, Elieh-Ali-Komi D, Criado PR, Peter J, Kolkhir P, et al. How infection and vaccination are linked to acute and chronic urticaria: a special focus on COVID-19. Viruses. 2023;15:1585.
39. Royer PY, Zayet S, Jacquin-Porretaz C, Kadiane-Oussou NJ, Toko L, Gendrin V, et al. Angioedema and COVID-19: a new dermatological manifestation? Infect Dis Rep. 2021;13:23–5.
40. Cugno M, Consonni D, Lombardi A, Bono P, Oggioni M, Uceda Renteria S, et al. Increased risk of urticaria/angioedema after BNT162b2 mRNA COVID-19 vaccine in health care workers taking ACE inhibitors. Vaccines. 2021;9:1011.
41. Levi M, van der Poll T. Two-way interactions between inflammation and coagulation. Trends Cardiovasc Med. 2005;15:254–9.
42. Vaswani SK, Plack RH, Norman PS. Acute severe urticaria and angioedema leading to myocardial infarction. Ann Allergy Asthma Immunol. 1996;77:101–4.
43. Pontillo D, Capezzuto A, Castellani R. Acute generalized urticaria leading to acute myocardial infarction. Angiology. 2000;51:89–90.
44. Yaegashi T, Nakamura Y, Sakagami S, Saeki T, Omi W, Ikeda K. Acute myocardial infarction following food-dependent exercise-induced anaphylaxis. Intern Med. 2011;50:451–4.
45. Connor S, Child N, Burdon-Jones D, Connor A. Cardiac arrest secondary to type 2 Kounis syndrome resulting from urticaria and angioedema. Emerg Med J. 2010;27:568–9.
46. Berth-Jones J, Hutchinson PE, Wicks AC, Mitchell VE. Chronic urticaria with angio-oedema controlled by warfarin. BMJ. 1988;297:1382–3.
47. Barlow RJ, Greaves MW. Warfarin in the treatment of chronic urticaria/angio-edema. Br J Dermatol. 1992;126:415–6.
48. Chua SL, Gibbs S. Chronic urticaria responding to subcutaneous heparin sodium. Br J Dermatol. 2005;153:216–7.
49. Asero R, Tedeschi A, Cugno M. Heparin and tranexamic acid therapy may be effective in treatment-resistant chronic urticaria with elevated D-dimer: a pilot study. Int Arch Allergy Immunol. 2010;152:384–9.
50. Takahagi S, Shindo H, Watanabe M, Kameyoshi Y, Hide M. Refractory chronic urticaria treated effectively with the protease inhibitors, nafamostat mesilate and camostat mesilate. Acta Derm Venereol. 2010;90:425–6.

51. Meyer-De Schmid JJ, Neuman A. Treatment of chronic urticaria with heparin. Bull Soc Fr Dermatol Syphiligr. 1952;59:286–7.
52. Parslew R, Pryce D, Ashworth J, Friedmann PS. Warfarin treatment of chronic idiopathic urticaria and angio-oedema. Clin Exp Allergy. 2000;30:1161–5.
53. Mahesh PA, Pudupakkam VK, Holla AD, Dande T. Effect of warfarin on chronic idiopathic urticaria. Indian J Dermatol Venereol Leprol. 2009;75:187–9.

Table 1. Sample description and examined factors/characteristics.

Variable	Mean	*SD*	Minimum	Maximum	Median	IQR
Age (years)	47.0	13.9	21	79	45	35.8–58.3
Factor VII (% of activity)	102.8	24.0	11	151	104	86.5–119.3
Fibrinogen (g/L)	3.6	0.7	2.3	5.2	3.5	3.2–4.1
D-dimer (FEU/L)	0.37	0.37	0.13	2.11	0.29	0.19–0.37

SD = standard deviation, IQR = interquartile range.

Table 2. Comparison of the prevalence of elevated coagulation factors between the three study groups.

	Level	HCs (n = 29)	CSU ± AE (n = 15)	AE (n = 14)	Sum	p	V
Factor VII, *n* (%)	Normal	9 (31%)	4 (26.7%)	1 (7.1%)	14 (24.1%)		
	Increased	20 (69%)	11 (73.3%)	13 (92.9%)	44 (75.9%)	0.222	0.228
Fibrinogen, *n* (%)	Normal	27 (93.1%)	13 (86.7%)	12 (85.7%)	52 (89.7%)		
	Increased	2 (6.9%)	2 (13.3%)	2 (14.3%)	6 (10.3%)	0.687	0.114
D-dimer, *n* (%)	Normal	28 (96.6%)	11 (73.2%)	13 (92.9%)	52 (89.7%)		
	Increased	1 (3.4%)	4 (26.7%)	1 (7.1%)	6 (10.3%)	0.051	0.320

HCs = healthy controls, CSU = chronic spontaneous urticaria, AE = angioedema, V = effect size (Cramer's V), p = significance level according to χ^2 test.

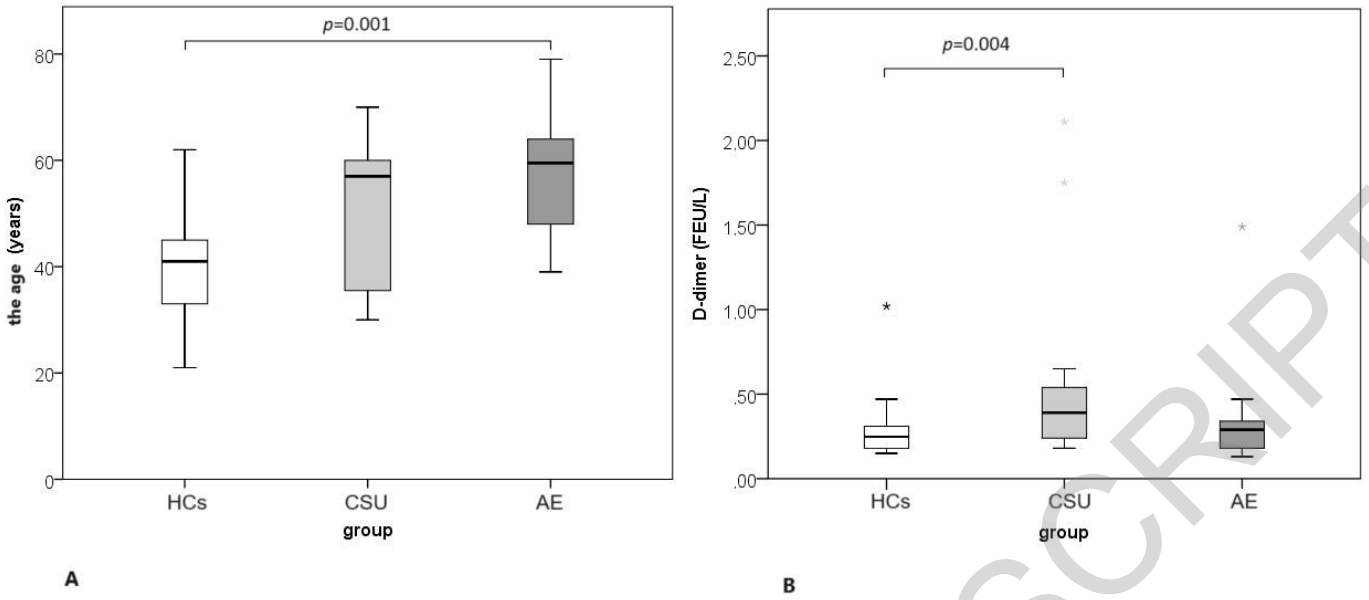

Figure 1. Comparison of age (A) and D-dimer values (B) among the three study groups. Asterisks show extreme values. Groups connected by a horizontal line significantly differ statistically, accounting for Bonferroni correction ($p \leq 0.017$). HCs = healthy controls, CSU = chronic spontaneous urticaria, AE = angioedema.

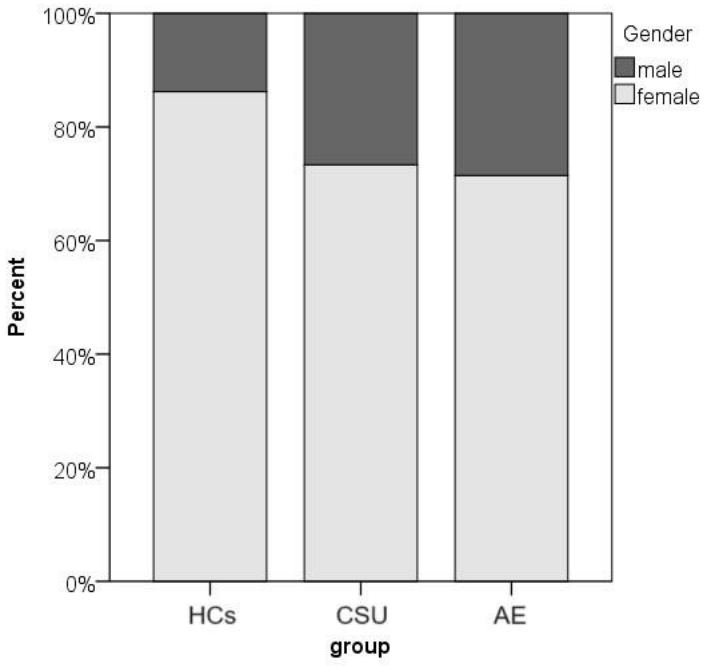

Figure 2. Data on participants' sex in the three study groups.

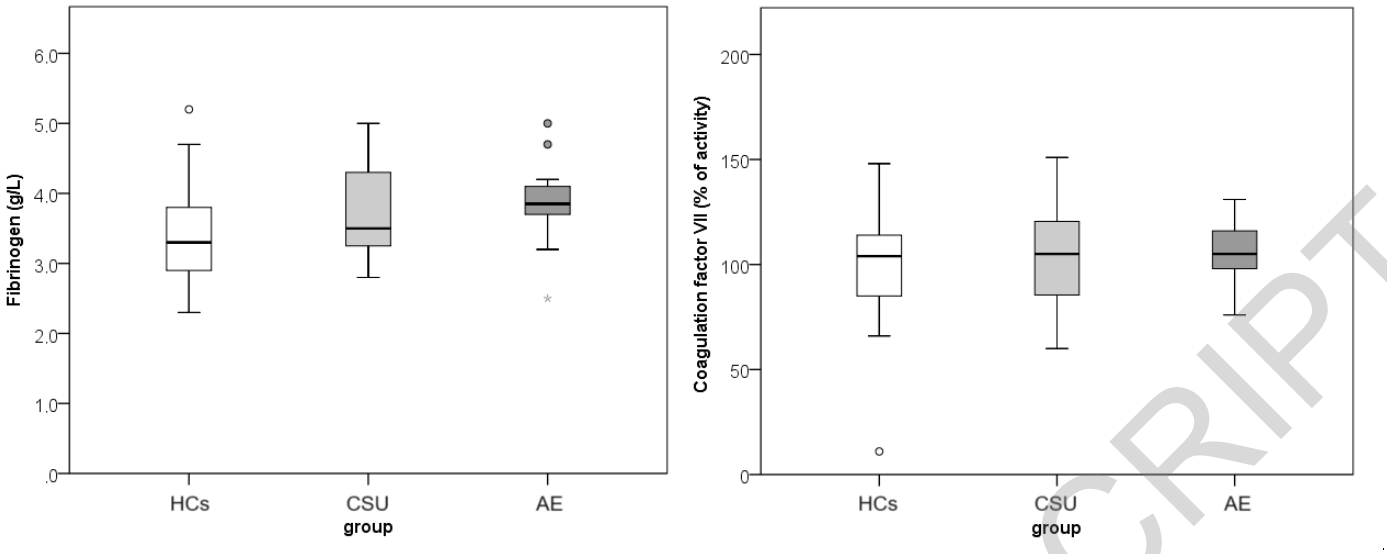

Figure 3. Comparison of fibrinogen and coagulation factor VII between the three study groups. Circles show outliers; stars show extreme values. HCs = healthy controls, CSU = chronic spontaneous urticaria, AE = angioedema.