Elevated thrombin generation and factor VIII activity during angioedema attack in patients with hereditary C1 inhibitor deficiency

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**Introduction** Hereditary angioedema (HAE) is a rare autosomal dominant disease caused by the deficiency of C1 inhibitor (C1INH). This HAE due to C1 inhibitor deficiency (C1INH-HAE) manifests as subcutaneous and submucosal angioedema, which can affect any part of the body.1

The deficiency of C1INH1,2 disturbs the complement cascade, coagulation, kinins, and fibrinolysis and results in an uncontrolled release of bradykinin, the main mediator in C1INH-HAE.3 Previous studies supported the involvement of blood coagulation and fibrinolysis in the pathophysiology of C1INH-HAE despite the absence of clinically relevant thrombosis and hemostatic disorders.4,5

To our knowledge, there have been no reports on the properties of plasma fibrin clot in relation to thrombin formation in this disease despite evidence on their role in several pathologies.5,6

We sought to evaluate thrombin generation and fibrinolytic potential along with factor VIII and fibrin clot density in patients with C1INH-HAE during remission and acute angioedema attack.

**Patients and methods** Patients The study included 15 adult patients with C1INH-HAE in remission and during angioedema attacks. The control group included 15 healthy individuals matched for age, sex, and body mass index. The diagnosis of C1INH-HAE was established on the basis of the patient and family history, on the examination during attacks of angioedema, and reduced C1INH antigen as well as C1INH activity below 50% of the reference values.1 The study was performed during remission (no attacks for at least 2 weeks) and in the first 3 to 5 hours of acute angioedema attack. Patients with comorbidities and those receiving any long-term medication (including tranexamic acid or danazol) were ineligible.

The ethics committee of Jagiellonian University in Kraków approved the study and patients signed a written informed consent form. The study was carried out in accordance with the Declaration of Helsinki and its amendments.

**Laboratory investigations** Blood samples (vol/vol, 9:1 of 3.2% trisodium citrate) were spun at 2000 g for 10 minutes, and the supernatants were aliquoted and stored at a temperature of −80°C. Plasma levels of the C1INH antigen (aC1INH) were measured using nephelometry (Siemens Healthcare Diagnostics GmbH, Marburg, Germany). The functional activity of C1INH was determined using the functional chromogenic assay Berichrom C1INH (Siemens Healthcare Diagnostics). All patients had reduced serum C1INH levels (mean, 0.07 g/l; reference range, 0.21–0.39 g/l) and functional activity of C1INH (mean functional activity of C1INH, 26.0%; reference range, 70%–130%). Plasma levels of factor VIII (FVIII; based on activated partial thromboplastin time) were evaluated using the Behring Coagulation System (Siemens Healthcare Diagnostics). ELISA test kits were used to measure thrombin–antithrombin complexes (TAT; Enzygnost TAT micro, Siemens Healthcare Diagnostics).

**Fibrin clot analysis** Plasma fibrin clot permeability was determined as described previously.7 The permeation coefficient (Kp), which indicates the average size of pores formed in the fibrin network (with low values indicating a tightly packed fibrin structure), was calculated from the equation: Kp = Q × L × η/t × A × Δp, where Q is the flow rate in time, L is the length of a fibrin gel, η is the viscosity of liquid (in poise), t is percolating time, A is the cross-sectional area (in cm²), and Δp is a differential pressure (in dyne/cm²).
During attack Categorical variables were median [IQR], Patients with C1INH-HAE did not dif
240 (223–296) mg/dl vs 235 [215–287] mg/dl and 3.68 [2.61–8.71] µg/l vs 3.03 [2.39–4.09] µg/L respectively. Patients with C1INH-HAE were characterized by 35% higher FVIII levels (median [IQR], 139% [129%–149%] vs 103% [92%–107%]; P <0.05), 66.4% higher endogenous thrombin potential (median [IQR], 1994 [1719–2203] nM×min vs 1198 [989–1298] nM×min; P <0.05), and 106% higher peak thrombin compared with controls (median [IQR], 305 [280–341] nM vs 148 [117–182] nM; P <0.05). In remission, K was lower by 19% (median [IQR], 6.05 [4.81–7.92] × 10⁻⁶ cm²/s vs 7.47 [6.62–9.01] × 10⁻⁶ cm²/s; P <0.05), while clot lysis time did not differ from the controls (median [IQR], 95 [90–112] min vs 95 [85–105] min; P >0.05). There were no associations of thrombin generation or fibrin clot properties with age, body mass index, or C1INH levels and activity in C1INH-HAE patients during remission.

Patients with C1INH-HAE during the attack compared with remission had 91.7% higher fibrinogen levels, 325.8% higher TAT levels, and 11.5% increased FVIII activity (table 1). During the attack, FVIII levels were associated with peak thrombin (r = 0.55, P = 0.04), while a similar association in remission tended to be significant (r = 0.5, P = 0.069). No other associations were observed for FVIII activity and fibrin clot properties.

Of note, we have not found differences in TAT levels between patients with C1INH-HAE and with erythema marginatum (n [%], 8 [53.3]) and those without erythema (n [%], 7 [46.7]) regardless of whether the patients were in remission (median [IQR], 6.00 [2.91–11.52] vs 3.68 [2.46–7.27] µg/l; P = 0.39) or during the attack (median [IQR], 39.74 [15.24–90.09] vs 7.82 [4.14–47.33] µg/l; P = 0.12).

Discussion Our study is the first to show elevated FVIII activity and increased thrombin generation reflected by TAT and in vitro thrombin generation potential in patients with C1INH-HAE. However, our results are based on a small group of patients and should be confirmed in a larger study that would also assess coagulation factors (especially von Willebrand factor) and their relationship with the duration and severity of an edema attack. Recently, increased thrombin generation assessed by CAT has been shown in patients with a chronic urticaria and a history of angioedema compared with patients with urticaria alone.10

An association between increased thrombin peak, elevated TAT, and FVIII levels may explain high D-dimer levels in patients with edema attacks, which are considered a biomarker of blood coagulation activation and fibrinolysis.5 Interestingly, increased FVIII activity in patients with C1INH-HAE during an angioedema attack and in remission is a novel observation. Recent studies have suggested the possible active involvement of

**TABLE 1** Characteristics of patients with hereditary angioedema due to C1 inhibitor deficiency during remission and attack

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In remission</td>
</tr>
<tr>
<td>Age, y</td>
<td>36 (29.5–56.5)</td>
</tr>
<tr>
<td>Female sex, n [%]</td>
<td>11 (73.3)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.6 (20.3–28.6)</td>
</tr>
<tr>
<td>Fibrinogen, mg/dl</td>
<td>240 (223–296)</td>
</tr>
<tr>
<td>Factor VIII, %</td>
<td>139 (129–149)</td>
</tr>
<tr>
<td>Fibrin clot permeability (Kⱼ) ×10⁻⁴ cm²</td>
<td>6.05 (4.81–7.92)</td>
</tr>
<tr>
<td>Clot lysis time, min</td>
<td>95.0 (89.5–111.7)</td>
</tr>
<tr>
<td>Lag time, min</td>
<td>2.94 (2.78–3.81)</td>
</tr>
<tr>
<td>Endogenous thrombin potential, nM×min</td>
<td>1994 (1719–2203)</td>
</tr>
<tr>
<td>Peak thrombin, nM</td>
<td>305 (280–341)</td>
</tr>
<tr>
<td>Time to peak thrombin, min</td>
<td>6.95 (6.29–7.5)</td>
</tr>
<tr>
<td>Thrombin–antithrombin complex, µg/l</td>
<td>3.68 (2.61–8.71)</td>
</tr>
</tbody>
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Data are presented as median (interquartile range) unless otherwise indicated.

a P <0.05 compared with patients in remission

Clot lysis time was measured using the assay by Pieters et al. It was defined as the time from the midpoint of the clear-to-maximum-turbid transition, which represents clot formation, to the midpoint of the maximum-turbid-to-clear transition.

**Calibrated automated thrombogram** Thrombin generation kinetics were measured with a calibrated automated thrombogram (CAT; Thrombinoscope BV, Maastricht, the Netherlands) according to the manufacturer’s instructions. Each plasma sample was analyzed in duplicate. The maximum concentration of thrombin formed during the recording time was described as the thrombin peak and the area under the curve represented endogenous thrombin potential.

**Statistical analysis** Categorical variables were analyzed by the Pearson χ² test. Continuous variables were expressed as median and interquartile range. Normality of the data was assessed using the Shapiro–Wilk test. The groups were compared using the t test for normally distributed continuous variables, the Mann–Whitney test and the Wilcoxon rank test were used. Associations between variables were assessed by the Spearman rank correlation analysis. P values of less than 0.05 were considered significant.

**Results** Patients with C1INH-HAE did not differ from age-, sex-, and body mass index–matched controls (all P >0.05). Eight patients (53.3%) had a positive family history of C1INH-HAE. The mean age of HAE onset was 9.9 years (range, 3–22 years). In 8 patients, angioedema attacks were preceded by erythema marginatum.

In remission, patients with C1INH-HAE had similar fibrinogen and TAT levels as controls (median [interquartile range (IQR)], 240 [223–296] mg/dl vs 235 [215–287] mg/dl and 3.68 [2.61–8.71] µg/l vs 3.03 [2.39–4.09] µg/L respectively). Patients with C1INH-HAE were characterized by 35% higher FVIII levels (median [IQR], 139% [129%–149%] vs 103% [92%–107%]; P <0.05), 66.4% higher endogenous thrombin potential (median [IQR], 1994 [1719–2203] nM×min vs 1198 [989–1298] nM×min; P <0.05), and 106% higher peak thrombin compared with controls (median [IQR], 305 [280–341] nM vs 148 [117–182] nM; P <0.05). In remission, K was lower by 19% (median [IQR], 6.05 [4.81–7.92] × 10⁻⁶ cm²/s vs 7.47 [6.62–9.01] × 10⁻⁶ cm²/s; P <0.05), while clot lysis time did not differ from the controls (median [IQR], 95 [90–112] min vs 95 [85–105] min; P >0.05). There were no associations of thrombin generation or fibrin clot properties with age, body mass index, or C1INH levels and activity in C1INH-HAE patients during remission.

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some blood clotting factors such as FXII and tissue factor–FVIIa complex\(^4,\,^11\) in the pathomechanism of C1INH-HAE.

We observed no difference in plasma fibrinolytic potential and fibrin clot density between patients with C1INH-HAE (regardless of remission or attack) and controls, despite elevated thrombin formation markers in the former group. This could explain, at least in part, the absence of thrombotic manifestations despite very high D-dimer levels found in patients with severe HAE attacks.\(^5\)

In most cases, efficient fibrinolysis seems to protect against thrombosis. However, high thrombin formation might suggest clinical benefits from thromboprophylaxis in some patients with high D-dimer levels during a severe attack.

During attack, contact system activation results in increased F1+F2 prothrombin fragment levels and shortening of activated partial thromboplastin time,\(^4\) which indicates enhanced thrombin generation due to FXII activation. Some authors\(^4,\,^11\) reported that during attack, FVIIa levels are increased, suggesting that self-limiting thrombin generation may be due to the activation of the tissue factor pathway. This phenomenon under bradykinin control as suggested by Dong et al\(^11\) deserves further research. Thrombin generation during attack activates thrombin activatable fibrinolysis inhibitor, which may indicate its effect on the regulation of fibrinolysis in this clinical setting. The involvement of endothelial activation in the pathogenesis of C1INH-HAE is in line with the study by Kajdácsi et al,\(^12\) who showed a significantly increased activity of von Willebrand factor.

Our study shows that thrombin generation related with increased FVIII activity rather than impaired fibrinolytic potential may be involved in modulating angioedema attacks in patients with C1INH-HAE. However, the major study limitation was a small sample size, thus the presented data should be interpreted with caution. In order to fully understand the pathophysiology of C1INH-HAE as well as the role of thrombin formation, blood clotting, fibrinolysis, and endothelial damage in this disease, further investigation on larger groups with long-term follow-up is required.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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REFERENCES