

Short communication

Plasma biomarkers of acute attacks in patients with angioedema due to C1-inhibitor deficiency

Background: C1-inhibitor (C1-INH) deficiency leads to recurrent attacks of mucocutaneous edema and may be inherited (hereditary angioedema [HAE]) or acquired (acquired angioedema [AAE]), which have the same clinical picture characterized by angioedema involving the skin, gastrointestinal tract, and larynx. Although cutaneous swelling is evident, abdominal angioedema is still a diagnostic challenge and attacks can mimic surgical emergencies. There is currently no laboratory marker for identifying angioedema attacks.

Objective: As coagulation and fibrinolysis are activated during angioedema attacks, we assessed if plasma measurements of prothrombin fragment F1 + 2 (marker of thrombin generation) and D-dimer (marker of fibrin degradation) can be useful for the diagnosis of angioedema because of C1-INH deficiency, especially in case of hidden locations as abdominal attacks.

Methods: In addition to complement, we measured plasma levels of F1 + 2 and D-dimer in 28 patients with C1-INH deficiency during acute attacks and remission, 35 patients without C1-INH deficiency during abdominal colics, and 20 healthy subjects.

Results: Plasma F1 + 2 levels were higher in patients with C1-INH deficiency during remission than in healthy controls ($P = 0.001$), and further increased during cutaneous and abdominal attacks ($P = 0.0001$); patients without C1-INH deficiency had normal F1 + 2 levels during abdominal colics. Plasma D-dimer levels were higher in patients with C1-INH deficiency during remission than in controls ($P = 0.012$) and increased during angioedema attacks, reaching higher levels than in patients without C1-INH deficiency during colics ($P = 0.002$).

Conclusions: During acute angioedema attacks, patients with C1-INH deficiency have high prothrombin fragment F1 + 2 and D-dimer levels, the measurement of which may have an important diagnostic value.

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Angioedema because of C1-inhibitor (C1-INH) deficiency, which may be hereditary (hereditary angioedema [HAE]) or acquired (acquired angioedema [AAE]), is characterized by episodic swellings of subcutaneous tissues, bowel walls, and upper airways. HAE is associated with C1-INH deficiency (type I) or dysfunction (type II), caused by mutations in one of the two C1-INH alleles, whereas AAE is characterized by a marked increase in C1-INH catabolism because of a concomitant, more often lymphoproliferative, disease and/or autoantibodies to C1-INH. HAE and AAE have the same symptoms of recurrent cutaneous edema affecting the face, extremities, trunk, and genitals. Involvement of the gastrointestinal tract leads to bowel sub-occlusion with severe pain, vomiting, and diarrhea; laryngeal edema can be life-threatening if it is not properly treated (1).

Diagnosing recurrent abdominal angioedema is hampered by the absence of objective findings and the lack

of laboratory markers identifying an ongoing angioedema attack, and is therefore often uncertain despite detailed descriptions of the symptoms and course of abdominal attacks (2). This uncertainty generates difficulties in therapeutic decision making, and so patients with C1-INH deficiency and acute abdominal attacks still undergo exploratory laparotomy, which could be avoided if a correct diagnosis could be made otherwise. Furthermore, the existence of plasma biomarkers, confirming the presence of angioedema, would facilitate the evaluation of treatment efficacy during clinical trials.

Specific diagnostic tests can be sought by looking at the pathophysiological mechanisms involved in the acute attacks of angioedema. C1-INH has a broad spectrum of activities; it inhibits the complement, contact, and fibrinolytic systems. Its deficiency renders the contact system and fibrinolysis unstable (3, 4). Various lines of evidence

obtained in HAE patients indicate that the contact system is activated during acute attacks, thus leading to the generation of bradykinin, which mediates increased vasopermeability and causes angioedema (5–7); furthermore, the bradykinin type-II receptor blockade seen in C1-INH knockout mice reverses the permeability defect (8). Activation of the fibrinolytic system leading to the generation of plasmin is observed during acute attacks in HAE (4), and during attacks and remission in AAE (9). *In vitro* data indicate that plasmin potentiates the generation of bradykinin by kallikrein on high molecular weight kininogen (10). Finally, the activation of the coagulation cascade during acute attacks leads to the generation of thrombin, which may contribute towards increasing vasopermeability (11).

The aim of this study was to investigate whether two commonly available plasma markers of thrombin generation (prothrombin fragment F1 + 2) and of fibrin degradation (D-dimer) (12) can be used diagnostically to distinguish angioedema attacks because of C1-INH deficiency, especially in case of hidden locations as abdominal attacks.

Materials and methods

Patients

We studied 28 patients with C1-INH deficiency (14 men and 14 women aged 49.3 ± 15.8 [mean $\pm$ SD] years, range 24–84), 23 of whom had HAE (21 type I and two type II), and five AAE.

Seven HAE and two AAE patients (one man and eight women aged 24–75 years) were studied during 10 acute abdominal attacks, five HAE and two AAE patients (two men and five women aged 33–84 years) were studied during 13 cutaneous attacks, and two AAE patients (one 70-year-old man and one 84-year-old woman) were studied during five oral mucosa attacks (Table 1). The diagnosis of the abdominal attack in the patients with known C1-INH deficiency was based on the presence of acute abdominal pain with or without vomiting and diarrhea, the absence of any other causes of abdominal pain, and prompt reversal upon the infusion of C1-INH (in HAE patients) or

tranexamic acid. All of the patients underwent routine laboratory tests and abdominal ultrasound examinations. None of our patients with abdominal attacks presented with cutaneous lesions at the time of the study.

The control groups consisted of 20 healthy subjects (10 men and 10 women with a mean age of 47.5 ± 15.7 years, range 24–79) and 35 patients without C1-INH deficiency experiencing acute abdominal pain (10 men and 25 women with a mean age of 44.3 ± 17.1 years, range 20–84), who were diagnosed as having gastroenteritis, gastritis, functional dyspepsia, irritable bowel syndrome, fecal impaction, cholelithiasis, mild pancreatitis, urinary colic, subacute appendicitis, dysmenorrhea, or abdominal adhesions.

Blood samples were collected using sodium citrate 3.8% as anti-coagulant. The samples were centrifuged at 2000 g for 20 min at room temperature, and the plasma was divided into aliquots, frozen, and stored at -80°C until testing.

All of the subjects gave their informed consent before participating in the study, the protocol of which was approved by our Institutional Review Board.

Methods

Plasma prothrombin fragment F1 + 2 was measured using an enzyme-linked immunosorbent assay (ELISA; Enzygnost F1 + 2; Behring Diagnostics GmbH, Frankfurt, Germany) with intra- and inter-assay coefficients of variation (CVs) of 5% and 8%.

Plasma D-dimer was measured by means of an ELISA (Zymutest D-dimer, Hyphen BioMed, Neuville-sur-Oise, France) with intra- and inter-assay CVs of 10% and 15%.

Functional C1-INH was measured using a chromogenic assay (Technochrom C1-inhibitor, Technoclone GmbH, Vienna, Austria), and C1-INH, C3, and C4 antigens by means of radial immunodiffusion (RID) (NOR-Partigen, Behring, Marburg, Germany).

Statistics

Results are reported as mean $\pm$ SEM and were log-transformed before analysis. Student's *t* test for unpaired values was used to assess statistical significance of differences. As multiple comparisons have been performed, the Bonferroni correction of *P*-values was carried out. The data were analyzed using the spss statistical package, version 15.00 (SPSS Inc., Chicago, IL, USA).

Results

All 28 angioedema patients showed functional C1-INH deficiency ($<40\%$ of normal) and C4 antigen deficiency ($<56\%$ of normal); C1-INH antigen levels were $<39\%$ of normal in the patients with type-I HAE and those with AAE, and between 54% and 200% of normal in those with type-II HAE. There was no significant difference in these measurements between the periods of acute attacks and remission.

As shown in Fig. 1, plasma F1 + 2 levels were higher in patients with C1-INH deficiency during remission (332 ± 74 pmol/l [mean $\pm$ SEM]) than in normal subjects (167 ± 21 pmol/l) ($P = 0.01$), and further increased during acute abdominal (1053 ± 355 pmol/l) and nonabdominal (1631 ± 236 pmol/l) attacks of angioedema ($P = 0.0001$ in both cases). The patients

Table 1. Angioedema attacks by location in 12 of 23 patients with hereditary angioedema (HAE) and in five patients with acquired angioedema (AAE)

	Patients with C1-inhibitor deficiency	Abdominal attacks		Cutaneous attacks		Oral attacks	
		No. of patients	No. of attacks	No. of patients	No. of attacks	No. of patients	No. of attacks
HAE	23	7	7	5	6†	0	0
AAE	5	2	3‡	2	7§	2	5¶

†A HAE patient was studied during two cutaneous attacks.

‡An AAE patient was studied during two abdominal attacks.

§One of the two AAE patients was studied during four cutaneous attacks and the other during three.

¶One of the two AAE patients was studied during two oral attacks and the other during three. The last patient had been previously studied during an abdominal attack.

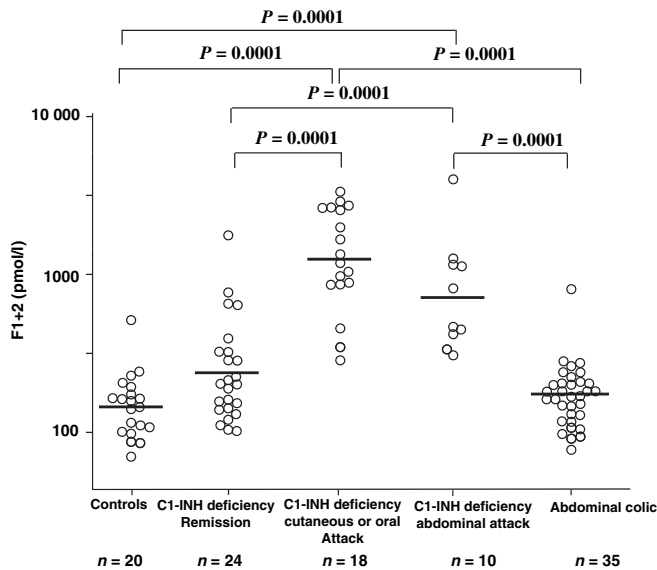


Figure 1. Plasma prothrombin fragment F1 + 2 levels (pmol/l) in healthy subjects (controls), patients with C1-INH deficiency during remission and during angioedema attacks (cutaneous/oral or abdominal), and in patients without C1-INH deficiency during abdominal colics. The horizontal lines indicate mean values.

without C1-INH deficiency had normal levels of F1 + 2 during abdominal colics (189 ± 20 pmol/l).

Figure 2 shows that plasma D-dimer levels were higher in patients with C1-INH deficiency during remission (1547 ± 566 ng/ml) than in normal controls (346 ± 33 ng/ml) ($P = 0.002$), and nonsignificantly increased during abdominal (4487 ± 2094 ng/ml) and nonabdominal attacks (2218 ± 958 ng/ml). The patients without C1-INH deficiency had significantly lower D-dimer levels (557 ± 75 ng/ml) during abdominal colics than those of the angioedema patients during abdominal attacks ($P = 0.0001$) and cutaneous or oral attacks ($P = 0.002$).

Discussion

As expected, functional plasma C1-INH levels in our HAE patients were 10%–40% of normal levels, far below the 50% of normal that would be predicted for an autosomal dominant trait. As previously demonstrated, the lower C1-INH levels of HAE patients are because of increased consumption (13), and possibly downregulation of the normal allele. The patients with acquired C1-INH deficiency also had functional plasma C1-INH levels that were 10%–40% of normal because of increased consumption because of the presence of lymphoproliferative diseases and/or autoantibodies (14). All of the patients with C1-INH deficiency had low C4 levels ($< 56\%$) because of consumption as a result of the activation of the classic complement pathway. The fact that there was no

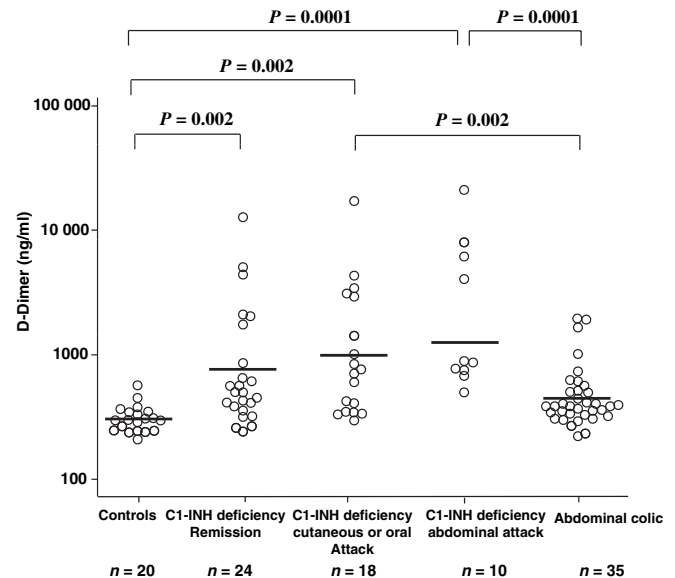


Figure 2. Plasma D-dimer levels (ng/ml) in healthy subjects (controls), patients with C1-INH deficiency during remission and during angioedema attacks (cutaneous/oral or abdominal), and in patients without C1-INH deficiency during abdominal colics. The horizontal lines indicate mean values.

difference in either C1-INH or C4 levels between remission and acute attacks confirmed that they cannot be used as markers of ongoing angioedema.

As the coagulation cascade and fibrinolysis are activated during acute angioedema attacks in patients with C1-INH deficiency (4, 11), we evaluated plasma markers of thrombin generation (prothrombin fragment F1 + 2) and fibrin degradation (D-dimer) as possible markers of angioedema, and found that both were abnormally high during acute attacks of abdominal and nonabdominal angioedema in the patients with C1-INH deficiency, but low in the healthy subjects and in the patients with abdominal colic in the absence of C1-INH deficiency.

Given the clear-cut difference in plasma F1 + 2 levels between our C1-INH deficient patients during attacks and the healthy controls, and the low levels found in the patients with abdominal colic but without C1-INH deficiency, it is possible to propose the measurement of F1 + 2 as a means of identifying an angioedema attack, and low levels of F1 + 2 seem to have a high negative predictive value. D-dimer levels, which have been extensively used as a negative prognostic marker in venous thromboembolic diseases (15), showed a trend that was consistent with the F1 + 2 results, but the considerable overlap of the measurements made during the periods of remission and acute attacks lessens their clinical relevance. Although the high dispersion of values of the plasmatic markers observed in our patients calls for a cautious interpretation of the findings, our results indicate that F1 + 2 levels can be proposed as a plasma marker of acute angioedema attacks in C1-INH deficient

patients, and that D-dimer levels may have supportive value. The use of these parameters is of particular interest as it can exclude angioedema because of C1-INH deficiency in patients presenting to emergency departments with abdominal pain of undetermined origin and, in patients with a diagnosis of HAE or AAE, it may distinguish an ongoing angioedema attack from abdominal pains due to other causes. Sometimes abdominal attacks are accompanied by a cutaneous lesion called erythema marginatum which, if present, may further support the diagnosis (2). Finally, plasma measurement

of F1 + 2 and D-dimer could be useful as objective means of assessing the presence of angioedema attacks in clinical trials, evaluating the efficacy of new treatments for patients with C1-INH deficiency.

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