



# Angioedema without urticaria: novel findings which must be measured in clinical setting

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## Purpose of review

Angioedema without urticaria is composed of an increasing subtype's variety and presents a challenging diagnosis. This review summarizes the subtypes recently described and subsequent new findings helpful within their classification.

## Recent findings

New methods to measure cleaved high molecular weight kininogen and activated plasma kallikrein have emerged as potential biochemical tests to identify bradykinin-mediated angioedema. Three new subtypes of hereditary angioedema (HAE) with normal C1 inhibitor were described in the past two years: HAE due to mutation in plasminogen gene, in kininogen gene, and in angiotensin-converting enzyme gene; implicating the fibrinolytic and contact systems, and the regulation of vasculature, respectively. The understanding of some mechanisms in angioedema has been improved, compatible to the dominant-negative for some C1 inhibitor variants; furthermore, the increased activation of truncated F12 mutants by plasma kallikrein; and the diminished binding of angiotensin-converting enzyme to its receptor.

## Summary

The validation of biomarkers for the contact system activation could be beneficial in differentiating bradykinin – from histaminergic-mediated angioedema. Currently, the available laboratorial tests are still somewhat restricted to the evaluation of the complement activation and the mediators of nonhistaminergic and nonbradykinin-mediated angioedema remain to be identified.

## Keywords

angioedema without urticaria, bradykinin, C1 inhibitor, F12, histaminergic

## INTRODUCTION

Angioedema is characterized by increased vascular permeability resulting in plasma extravasation of the subcutaneous and submucosal layers of skin [1]. Angioedema is a prevailing symptom in different diseases, frequently occurring in the presence of urticaria. However, recurrent angioedema without urticaria is considered a distinct disease with different hereditary angioedema (HAE) and acquired angioedema (AAE) types. Although 10 angioedema subtypes can be distinguished (Fig. 1), their clinical presentation is quite similar. The characteristic swellings from angioedema are self-limited, and commonly affect extremities, face, bowels, genitalia, and upper airways, and lasts for as much as 2–5 days with spontaneous remission [2]. Although subcutaneous swellings have been known to be very painful and disfiguring, bowels edema closely resembles acute abdomen, leading many patients to erroneous diagnosis to unnecessary surgeries [3]. Furthermore, inappropriate management of upper airways edema may result in death by asphyxiation. Angioedema is misdiagnosed because of

the overlapping symptoms with other diseases and the difficulty to distinguish the numerous angioedema subtypes. Here, we review the recent findings in angioedema focus on new potential biomarkers to improve disease classification and diagnosis.

## Bradykinin-mediated angioedema and the contact system

Regardless of the clinical presentation similarities with histamine-mediated angioedema, bradykinin-

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## KEY POINTS

- The ability to distinguish between bradykinin and histamine-mediated angioedema is crucial for the fast and correct diagnosis and management of angioedema without urticaria.
- The molecular diagnosis is the only available test to accurately identify hereditary angioedema with normal C1INH and mutations in *F12*, *PLG*, *ANGPT1*, and *KNG1* genes.
- The measurement of cleaved high molecular weight kininogen and total activated plasma kallikrein are potential biomarkers for the diagnosis of bradykinin-mediated angioedema.
- Patients with HAE due to C1 inhibitor mutations are potential candidates for gene therapy despite the dominant-negative *SERPING1* variants responsible for intracellular retention and low plasma levels of C1 inhibitor.

mediated angioedema patients are consequently not responsive to antihistamines, epinephrine and corticosteroids, and its occurrence is much lower when compared with histamine-mediated angioedema [4]. The capability to differentiate these two forms of angioedema is pivotal in the proper management of the disease, reducing its morbidity and mortality [5<sup>22</sup>]. In the contact system, bradykinin is a vasoactive nonapeptide belonging to kininogen amino acid sequence. This system is composed of two central

enzymes that circulate in plasma as zymogens, the coagulation factor XII, and the plasma kallikrein [6]. Factor XII is autoactivated by the interaction with negative surfaces such as platelet polyphosphate [7] and heparin [8]. Activated factor XII cleaves and activates plasma kallikrein, which hydrolysis high molecular weight kininogen (HMWK) releasing the bradykinin molecule. Free bradykinin binds to the transmembrane kinin B2 receptor constitutively expressed in endothelial cells, leading to the increase in vascular permeability [1]. The main regulator of the contact system is the C1 inhibitor, a serine protease inhibitor capable to inhibit a series of enzymes from the complement, fibrinolytic, coagulation, and contact system, like C1r, C1s, coagulation factors XI and XII, plasma kallikrein and plasminogen [9]. Therefore, dysfunctions in any of the main components of the contact system that can lead to an enhanced release of bradykinin are candidates to cause bradykinin-mediated angioedema, as observed in most of angioedema subtypes and in different animal models [10,11].

## Types of hereditary angioedema

### Hereditary angioedema with C1 inhibitor deficiency

HAE with C1 inhibitor (C1INH) deficiency (HAE-C1INH) (OMIM No. 606860) is an autosomal dominant disease caused by deleterious mutations in the gene encoding the C1INH, *SERPING1*, leading to

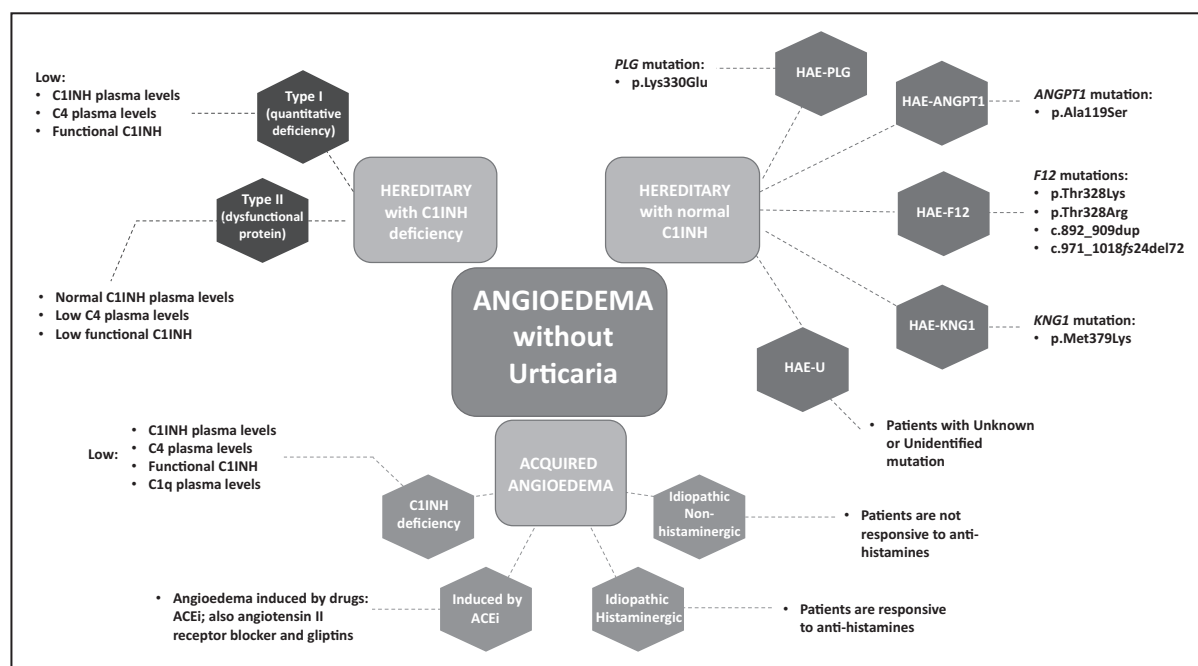


**FIGURE 1.** Angioedema without urticaria: subtypes and tools for diagnosis.

low quantitative C1INH plasma levels (HAE-C1INH type I) or dysfunctional C1INH (type II) [12<sup>■</sup>]. HAE-C1INH is the most common subtype of HAE affecting approximately 1:50 000 individuals worldwide [13].

The diagnosis of HAE-C1INH is based on inherent clinical symptoms, family history, and C1INH and C4 plasma measurements. In HAE-C1INH type I, both C1INH and C4 plasma levels are below 50%; and in HAE-C1INH type II, whilst C4 is low, C1INH plasma levels are normal or even high, and C1INH activity shall be measured to confirm low C1INH function [2].

Diverse studies investigated new biochemical and genetic biomarkers that would be able to clarify the variability of symptoms and predict attacks [14,15<sup>■</sup>,16,17]. There are evidence that the levels of C1INH in plasma may correlate to the risk of attacks [15<sup>■</sup>], but its routine measurement is still infeasible.

Over 500 mutations associated to HAE-C1INH are described in *SERPING1* [18], and approximately 6% corresponds to de-novo mutations according to a recently published study [19], in contrast to the 25% of de-novo mutations estimated over 20 years ago [20]. In approximately 5–10% of HAE-C1INH cases, no mutation in the coding region of *SERPING1* is identified [1,12<sup>■</sup>,19]. The genetic sequencing of *SERPING1* is not necessary for the HAE-C1INH diagnosis; however, the molecular diagnosis may be particularly helpful to distinguish between de-novo mutations and acquired angioedema forms when the family history is absent. Loules *et al.* [21] proposed a next-generation sequencing platform able to sequence *SERPING1* in large scale in combination with time and cost restrictions and savings. The penetrance of HAE-C1INH is high, with only about 5% of asymptomatic patients [13], but the frequency and severity of attacks can widely vary in a lifetime and from patient to patient, even among patients within the same family and equally affected by the same disease-causing mutation [1,22].

### **Hereditary angioedema with normal C1 inhibitor**

The term HAE with normal C1INH (HAE-nl-C1INH) was considered and recommended approximately 20 years ago to classify HAE families with the same clinical symptoms of HAE-C1INH, but with normal quantitative and functional C1INH [23]. In 2006, the first description of affected male patients [24], as well as the association of HAE-nl-C1INH with factor 12 mutation [25], initiated a new chapter in HAE classification and research. Currently, five subtypes

of HAE-nl-C1INH can be distinguished regarding their genetic cause: the HAE-nl-C1INH with mutations in the gene encoding the coagulation factor XII (*F12*; HAE-F12) [25], the plasminogen gene (*PLG*; HAE-PLG) [26], the angiopoietin-1 gene (*ANGPT1*; HAE-ANGPT1) [27], and the kininogen gene (*KNG1*; HAE-KNG1) [28<sup>■</sup>], apart from a group of patients in which the genetic cause of HAE is still unknown (HAE-U) (Fig. 1).

### **Hereditary angioedema with normal C1 inhibitor with F12 mutations**

A higher activation and/or activity of factor XII will result in more plasma kallikrein being activated and a consequent increase in bradykinin release. Four different mutations in *F12* had been identified [25,29,30]. All these mutations lie in a proline-rich region of factor XII heavy chain, encoded by exon 9 of *F12*. The changes p.Thr328Lys/Arg cause a loss of O-linked glycosylation increasing factor XII susceptibility for contact activation instead of the enzyme's activity [31]. Ivanov *et al.* [32<sup>■</sup>] demonstrated that p.Thr328Lys/Arg is susceptible to the cleavage by thrombin and factor XI, generating a truncated factor XII that is activated by plasma kallikrein with augmented catalytic efficiency.

### **Hereditary angioedema with normal C1 inhibitor with plasminogen gene mutations**

HAE-PLG was first identified in 2018, by whole-exome sequencing, in two families carrying the mutation p.Lys330Glu in an autosomal dominant inheritance pattern [26]. Afterward, more than 10 families have been described in different countries [33–35]. It is assumed that an increase in the conversion of plasminogen to plasmin is able to generate an augmented activation of the contact system, as plasmin also acts as an activator of factor XII. Nowadays, it is only known that plasminogen activity is normal in patient's plasma and the exact mechanism of p.Lys330Glu remains unknown [26,34]. Although a recent consensus in the use of genetics in management of HAE recommends only the sequencing of exon 9 of *F12* as a routine molecular diagnosis [12<sup>■</sup>], the increasing number of families being diagnosed with p.Lys330Glu in *PLG* build up evidences for a prevalence higher than expected. Whole-genome sequencing performed in 2820 healthy individuals only one woman with p.Lys330Glu mutation and a previous history of recurrent lip and tongue swellings diagnosed as idiopathic and nonresponsive to administered antihistamines [36].

### Hereditary angioedema with normal C1 inhibitor with angiopoietin-1 gene mutations

In 2018, a family with *ANGPT1* (c.807G>T, p.Ala119Ser) mutation was reported, affecting all symptomatic members [27]. Angiopoietin-1 regulates the integrity of the endothelial barrier function through multiple permeability-enhancing factors, including vascular endothelial growth factors (VEGF). The alteration p.Ala119Ser was shown to reduce the multimerization of angiopoietin-1 [27] and its ability to bind to the internal endothelial tunica of cell kinase-2 receptor in an apparent haploinsufficiency mechanics [37<sup>¶</sup>]. This modification provides a possible mechanism by which the vasculature of HAE-ANGPT1 patients is prone to an increased vascular permeability potentially mediated by different vasoactive factors, including bradykinin. A recent study including four HAE-ANGPT1 patients did not find any alteration in plasma levels of any of VEGF-A, VEGF-C, angiopoietin-1 and 2, cleaved kininogen, or secreted phospholipase A2 enzymes [38<sup>¶</sup>].

### Hereditary angioedema with normal C1 inhibitor with kininogen gene mutations

A recent article described a single HAE-nl-C1INH family in which the novel variant c.1136T>A was identified in exon 10 of *KNG1* in all the six symptomatic members, but in none of the healthy relatives [28<sup>¶</sup>]. This variation was predicted to be likely pathogenic by in-silico analysis and according to the American College of Medical Genetics 2015 guidelines. As reported by the authors, no abnormality was found in kininogen or plasma-kallikrein measurements. Although further studies are necessary to clarify the mechanism involved in this new HAE-nl-C1INH subtype, this amino acid change affects the N-terminal cleavage site in kininogen [28<sup>¶</sup>]. This variation could further generate a modified active peptide more resistant to degradation.

### Hereditary angioedema with normal C1 inhibitor of unknown cause

A huge number of patients present HAE symptoms, family history, normal biochemical parameters of C1INH and C4, and no identified mutation. Whole-exome sequencing allowed the discovery of new genes responsible for HAE-U in HAE-ANGPT1, HAE-KNG1, and HAE-PLG; notwithstanding, it is not part of the routine molecular diagnosis and thus the interpretation of new variants warrants further research investigation.

### Acquired angioedema with C1 inhibitor deficiency

Acquired C1INH deficiency patients have late onset of symptoms, no family history of angioedema, no genetic mutations in *SERPING1* and clinical symptoms resembling HAE-C1INH [39]. This clinical condition is frequently associated with the production of antibodies anti-C1INH and/or lymphoproliferative diseases such as monoclonal gammopathy of undetermined significance and non-Hodgkin lymphoma. These situations lead to the consumption of C1INH and, consequently, lack of control in the contact system. Epidemiological data of acquired C1INH deficiency suggests a prevalence of one case of AAE with C1INH deficiency for 8.8 cases of HAE-C1INH. Decreased concentrations of C4 and quantitative and functional C1INH occur in most cases as well as decreased C1q levels (which rarely occur in HAE-C1INH) [39,40]. In a recent study, Bork *et al.* [28<sup>¶</sup>] described that the angioedema episodes started more than one year before the diagnosis of the associated disease in 41% of patients, implying that late recurrent isolated angioedema episodes can be an indication for some lymphoproliferative disorders. When biochemical data are not strongly consistent, and no associated disease or auto-antibodies are detected, genetic sequencing of *SERPING1* may be necessary to exclude HAE-C1INH [39,40]. There are evidence that the assays quantifying C1INH/anti-C1INH complex are more sensitive than the recognition of C1INH autoantibodies itself by enzyme-linked immunosorbent assay. A recent study showed that the complex of C1INH/anti-C1INH was detected in 18/20 patients with acquired C1INH deficiency, whereas in only half of them (9/18) free anti-C1INH was detected [41<sup>¶</sup>]. Furthermore, they presented an association of immunoglobulin M C1INH/anti-C1INH complex measurement and very low C1q levels, whereas the presence of immunoglobulin G C1INH/anti-C1INH complex, free immunoglobulin M or free immunoglobulin G anti-C1INH were associated with normal to slightly low C1q [41<sup>¶</sup>].

### Angioedema induced by angiotensin-converting enzyme inhibitors

Angiotensin-converting enzyme is responsible for the conversion of angiotensin I in angiotensin II, but it is also one of the enzymes responsible for the degradation of bradykinin. Therefore, with this in mind, the use of angiotensin-converting enzyme inhibitors (ACEis) also reduces bradykinin degradation. Although limited to 1% of the patients under ACEi treatment develop angioedema, it is estimated

that 1/3 of the antihypertensive drugs prescribed in the United States were ACEi [42]. Hubers *et al.* [43] identified high bradykinin levels in the plasma of patients with angioedema induced by ACEi accompanied by low levels of angiotensin-converting enzyme (ACE) metabolites, reinforcing the role of bradykinin. However, a metaanalysis comparing the relevant randomized clinical trials of icatibant (bradykinin B2 receptor antagonist) efficacy in angioedema induced by ACEi treatment revealed no benefits of this drug over placebo [44<sup>¶</sup>]. Different studies failed in identifying a genetic cause for angioedema induced by ACEi, whereas a few polymorphisms have been hitherto suggested to be associated with its increased risk [45].

### Idiopathic histaminergic and nonhistaminergic acquired angioedema

Idiopathic histaminergic AAE is mediated by mast cell degranulation and histamine release; however, clinical presentation is similar to HAE and other AAE subtypes. Its diagnosis is defined by the response to antihistamines. A thought-provoking investigation reports an unquestionably high incidence of antihistamine resistance affecting 36% of 120 patients initially diagnosed with idiopathic histaminergic AAE [46]. In a cohort of 68 patients, the mean age at the onset of disease was approximately 40 years old, attacks affected mainly face, and the males were slightly more affected than women [4]. In the same study, tranexamic acid prophylaxis significantly reduced frequency and severity of attacks in 86.2% of patients with an initial dose of 1 g three times a day ( $P=0.0001$ ) [4], corroborating with previous studies reviewed by Bucher *et al.* [47].

Idiopathic nonhistaminergic AAE patients are defined with recurrent isolated episodes of angioedema despite high doses of antihistamines and no other identified associated agents. Recent reports showed improved patients after long-term use of omalizumab, effectively abolishing angioedema attacks [4,47,48]. Therefore, diverse mediators such as bradykinin, leukotrienes, platelet-activating factor and prostaglandins may be involved in this AAE subtype.

### Measurement of biomarkers in angioedema

Clinical evaluation of angioedema without urticaria is fundamental to establish differential diagnosis as described before. At this point, it is paramount to recognize that angioedema is a common condition. Nevertheless, laboratorial test can confirm or exclude hereditary angioedema but a careful analysis of the results has to be done. Differentiating bradykinin from histamine-mediated angioedema is of critical relevance.

Although bradykinin-mediated disease results mainly from disturb in contact system, complement evaluation has been used for diagnosis. Inappropriate handling of the samples leads to misdiagnosis and, as a consequence, mistaken approaches. Complement tests require serum or plasma samples. Centrifugation and adequate storage should be done on the day of collection if the tests are not performed immediately. Short term storage can be done at  $-20^{\circ}\text{C}$  (max. 3 months) or at  $-70^{\circ}\text{C}$  for long-term periods before analysis. Repeated freeze-thawing hampers functional tests and small aliquots can prevent it [49].

Measuring C4 levels is a reliable screen for C1INH deficiency and reduced C4 is found in the majority of patients with HAE, particularly during attacks [49,50]. It is important to keep in mind that a low antigenic C4 is only indicative of HAE-C1INH, but it is not always conclusive because of the high frequency of C4 null alleles in healthy individuals, associated with lower C4 levels [51]. It was described a high negative predictive value (96%) for C4 values, but the sensitivity of C4 for HAE is between 65 and 85% [50,52,53]. Normal serum C4 does not always exclude the diagnosis of HAE [50]. Patients with suggestive clinical manifestations should undergo testing antigenic C1 INH levels even with normal C4.

Approximately 85% of HAE-C1INH deficit are type I and 15% are related to dysfunctional protein (type II), thus antigenic C1INH could identify HAE-C1INH type I [13]. Functional C1INH is reduced in both types I and II but the tests have shown more variability because of the sensitivity to sample manipulation.

C1INH concentrations are obtained by radial immunodiffusion, turbidimetry, or nephelometry and the results generated are equivalent. Radial immunodiffusion for quantification is time consuming whereas the others are automated and quicker [54]. The choice of the selected method depends on local demands and costs. For example, centers without access to equipment to perform nephelometry or turbidimetry could follow with investigation using 'manual' tests as radial immunodiffusion. In addition, the acquisition of the equipment for few dosages could be cost wasting.

Although in most of the HAE-C1INH cases the pathogenic mutation in *SERPING1* occurs in heterozygosity, C1INH plasma levels are below 50% of the normal (20–30%), implying in a reduced expression or secretion of the C1INH transcribed by the normal allele. Haslund *et al.* [55<sup>¶¶</sup>] demonstrated that some of the abnormal C1INH transcribe by HAE-causing mutated *SERPING1* can aggregate intracellularly with the normal C1INH transcribed by the wild type allele. This dominant-negative mechanism helps to

explain the C1INH plasma levels below 50% found in many type I patients despite the presence of one normal allele. Nonetheless, the proposed mechanism was not reproduced in all the mutations studied [55<sup>\*\*\*</sup>].

The results of blood C1INH tests should be interpreted with caution in pregnant women with angioedema, because of the transitory decline in plasma C1INH levels during pregnancy, consequent to the expansion of plasma volume [2,49]. Also, evaluation for C1INH levels in infants less than one year could lead to misdiagnosis. Cord blood samples are often equivocal [56,57].

Functional C1INH represents the main test for HAE diagnosis but the obstacles related with handling samples, time of storage, and restricted offer of this test limit its use. A survey developed among Canadian physicians showed their distrust in the results of this assay, probably because of the mentioned factors [51]. Two types of tests are currently used to measure functional C1INH: ELISA detects complexes formed as a result of the activation of C1 but is not a direct measurement of inhibition (antibodies anti-C1INH binds to C1s/C1INH complexes) and the chromogenic C1INH functional assay measures the ability of C1INH to inhibit C1s activity in a solution phase assay [49]. A previous study found that the ELISA and chromogenic methods have similar positive predictive values, but the chromogenic method has a greater negative predictive value [58]. The sensitivity of chromogenic and ELISA assays for functional C1INH test was reported as 73 and 56%, respectively [50,59]. Functional C1INH should be analyzed with caution and the simultaneous evaluation of C4 and C1INH concentrations could be reliable when undertaken by a specialist [52].

Diagnosis of AAE is predominantly based on the same biochemical tests as HAE; C1INH protein concentration, C4 concentration and C1INH functional tests, but with the addition of anti-C1INH antibody testing and C1q concentrations. Radial immunodiffusion or ELISA tests are available for measuring C1q level, which is characteristically normal in hereditary form and reduced (approx. lower than 0.06 g/l) in acquired form of C1INH deficiency [49]. Similar to HAE, around 20% of patients have normal antigenic levels of C1INH, but reduced function [60]. In the evaluation of more than 70 patients with acquired angioedema identified auto-antibodies to C1INH in 68% of them. Median plasma levels of functional and antigenic C1INH, and antigenic C4 and C1q, were below 50% of normal. Plasma C1q levels were reported normal (i.e., >70%) in approximately 20% of the patients tested [39]. In summary, clinical history with late-onset angioedema and no

family history with possible variability in laboratory results can lead to the suspicion and diagnosis of AAE.

Considering the involvement of the fibrinolytic system in HAE-nC1INH, the levels of plasminogen activator inhibitor 2 were reported as deficient in patients with HAE-nC1INH [61], but this finding could not be reproduced in our patients [62] as well as by others [63].

Validated biomarkers of contact system activation could be helpful in differentiating mechanisms of angioedema. The kininogen cleavage or plasma kallikrein activities may increase the ability of diagnosing different types of HAE and other bradykinin-mediated angioedema [64]. Joseph *et al.* [61] developed an assay evaluating C1INH function by its binding to immobilized activated factor XII or plasma kallikrein. The advantage of this test is its ability to measure C1INH complexed with the relevant contact system proteases. These activation products are generated also during the in-vivo release of bradykinin, and therefore, they might prove as a relevant diagnostic parameter [61]. Considering the limitations to diagnose bradykinin-mediated angioedema with normal C1INH, this assay could represent a possible option. Recently, Lara-Marquez *et al.* [5<sup>\*\*\*</sup>] developed an assay based on activation of plasma kallikrein following ex-vivo stimulation of the contact system in plasma, discriminating histamine from bradykinin-mediated angioedema. HAE-C1INH, HAE-nC1INH and Idiopathic nonhistaminergic angioedema groups had significantly elevated kallikrein activity compared with Idiopathic histaminergic angioedema and nonswelling groups [5<sup>\*\*\*</sup>]. The main prophylactic therapies, introduced in the last years, are bradykinin targeted medications and this fact has to be considered for these types of assay. The tests evaluating contact system are not yet commercially available.

Other cleaved proteases could also contribute toward the diagnosis of functional C1INH reflecting contact system activation. The measurement of cleaved HMWK in the plasma of angioedema patients conceivably be a biomarker of the contact system activation. When analyzed by immunoblotting, cleaved kininogen correlates with the severity of attacks in HAE-C1INH patients and was significant higher when compared with controls [65,66]. However, cleaved HMWK requires immunoblotting, laborious methods and it is influenced by sample collection [38<sup>\*</sup>]. Bradykinin, the responsible for changing endothelial permeability, has a short life, therefore, being difficult to measure. Other immunoassays and liquid chromatography–mass spectrometry (LC–MS/MS) have been proposed as more reliable methods [67,68]. The current tests

seem to suffer a high variability regarding the presence or absence of protease inhibitors [38<sup>¶</sup>] and their sensitivity is limited to accurately distinguish patients during remission from controls [65–68].

## FINAL CONSIDERATIONS

New categories of angioedema have been illustrated and reported within the last 20 years; nevertheless, a body of patients are included as unknown HAE or idiopathic angioedema. Thus, the advancement of assays to evaluate and diagnose these thought-provoking patients will benefit and diminish misdiagnosis and therein understanding pathophysiology of the angioedema. On the alternate side, pharmacological treatment, which was not the current focus, has developed rapidly and also drove the groundbreaking knowledge pertaining to several topics of angioedema. Gene therapy is currently under development with successful restoration of C1INH secretion in fibroblasts within mice using lentiviral vectors encoding wild type *SERPING1* [69<sup>¶¶</sup>]. In conclusion, we still have underdeveloped identification of HAE, restricted laboratorial testing and exploring complement activation, nevertheless an increasing interest in angioedema without urticaria.

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## Conflicts of interest

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