Clinic Rev Allerg Immunol (2016) 51:140–151
DOI 10.1007/s12016-016-8539-6

"Nuts and Bolts" of Laboratory Evaluation of Angioedema

Henriette Farkas [1,2] · Nóra Veszeli [1,2] · Erika Kajdácsi [1] · László Cervenak [1] ·
Lilian Varga [1,2]

Published online: 3 May 2016
© Springer Science+Business Media New York 2016

Abstract Angioedema, as a distinct disease entity, often becomes a clinical challenge for physicians, because it may cause a life-threatening condition, whereas prompt and accurate laboratory diagnostics may not be available. Although the bedside diagnosis needs to be established based on clinical symptoms and signs, family history, and the therapeutic response, later, laboratory tests are available. Currently, only for five out of the nine different types of angioedema can be diagnosed by laboratory testing, and these occur only in a minority of the patient population. Hereditary angioedema with C1-inhibitor (C1-INH) deficiency type I can be diagnosed by the low C1-INH function and concentration, whereas in type II, C1-INH function is low, but its concentration is normal or even elevated. C1q concentration is normal in both forms. Acquired angioedema with C1-INH deficiency type I is characterized by the low C1-INH function and concentration; however, C1q concentration is also low, and autoantibodies against C1-INH cannot be detected. Complement profile of acquired angioedema with C1-INH deficiency type II is similar to that of type I, but in this form, autoantibodies against C1-INH are present. Hereditary angioedema due to a mutation of the coagulation factor XII can be diagnosed exclusively by mutation analysis of *FXII* gene. Diagnostic metrics are not available for idiopathic histaminergic acquired angioedema, idiopathic non-histaminergic acquired angioedema, acquired angioedema related to angiotensin-converting enzyme inhibitor, and hereditary angioedema of unknown origin; these angioedemas can be diagnosed by medical and family history, clinical symptoms, and therapeutic response and by excluding the forms previously described. Several potential biomarkers of angioedema are used to date only in research. In the future, they could be utilized into the clinical practice to improve the differential diagnosis, therapy, as well as the prognosis of angioedema.

Keywords Angioedema · Diagnosis · C1-inhibitor · Laboratory parameters · Biomarkers

Introduction

Established methods of laboratory diagnosis are currently available only for angioedema (AE), resulting from C1-inhibitor (C1-INH) deficiency, as well as for a specific type of hereditary angioedema (HAE) due to a mutation of the coagulation factor XII (FXII-HAE) [1]. Unfortunately, these diagnostic metrics are not available for the other acquired angioedema forms (AAE) such as idiopathic histaminergic acquired angioedema (IH-AAE), idiopathic non-histaminergic acquired angioedema (InH-AAE), acquired angioedema related to angiotensin-converting enzyme inhibitor (ACEI-AAE), and hereditary angioedema of unknown origin (U-HAE) [2]. Thus, these types can be diagnosed by indirect methods such as medical and family history, clinical symptoms, and therapeutic response or by excluding AEs with C1-INH deficiency and FXII-HAE.

✉ Henriette Farkas
farkas.henriette@med.semmelweis-univ.hu

1 Research Laboratory, 3rd Department of Internal Medicine, Semmelweis University, Kútvölgyi út 4, H-1125 Budapest, Hungary

2 Hungarian Angioedema Center, 3rd Department of Internal Medicine, Semmelweis University, Kútvölgyi út 4, H-1125 Budapest, Hungary

Diagnostic Algorithm of Angioedema

Clinically, establishing the diagnosis of cutaneous involvement in AE is straightforward. The typical non-pruritic, non-erythematous swelling can appear on the skin of the face, lips, neck, trunk, abdomen, external genitals, or lower extremities. Upper airway edema involving the mucosa of the mesopharynx (pharyngeal arcs, uvula, soft palate) or of the tongue can be recognized by inspection, using a spatula. The visualization of laryngeal or hypopharyngeal involvement requires ear-nose-throat (ENT) examination (indirect or direct laryngoscopy). When the latter is not available, upper airway edema can be diagnosed based on indirect signs only. The following are possible, subjective symptoms of upper airway edema (ranked by increasing severity): sore, scratchy, itchy throat; foreign body sensation ("something has stuck in my throat"); lump sensation in the throat; feeling of throat tightness; dysphagia; voice changes (high-pitched or hoarse voice, roughness of voice); resonant, "barky" cough; stridor; dyspnea; fear of suffocation; aphonia; inability to breathe, speak, or cough; anxiety; and agitation [3]. The intestinal wall is another typical, submucosal location of AE. Edema confined to the gastrointestinal mucosa is associated with colicky abdominal pain, nausea, vomiting, post-attack watery diarrhea, and occasionally, circulatory collapse resulting from hypovolemia. It is commonly accompanied also by limb edema [4]. Upon imaging or direct endoscopic examination, the abdominal mucosa reveals thickening of the intestinal wall, with or without free peritoneal fluid [5]. Abdominal CT and MRI reveal massive small bowel or colonic edema, prominent mesenteric vessels, thickened omentum, and moderate ascites [6]. The edema of the intestinal wall can be confirmed also by capsule endoscopy [7]. In rare instances, e.g., in colostomy patients, mucosal edema can be visible to the naked eye upon inspection [8]. No specific, "rapid test" exists to establish an accurate diagnosis immediately upon the onset of the angioedematous manifestations. In AE affecting the skin or the upper airway mucosa, the patient's response to "standard" acute therapy (i.e., administration of an antihistamine, glucocorticoid, and epinephrine) provides the initial diagnostic clue. If the swelling resolves in response to standard therapy, it should be assumed to be "allergic" *histamine*-mediated. The diagnostic evaluation of the latter is the same as that of AE accompanying urticaria [9]. Such cases with no identifiable allergen as a cause of edema formation may be regarded as IH-AAE. Often, the patient presents without acute manifestations; however, the medical history reveals recurrent episodes of subcutaneous and/or upper airway edema or abdominal symptoms of unclear etiology. When acute or recurrent subcutaneous and/or upper airway edema defies standard therapy, and if abdominal US reveals edema of the intestinal wall or free peritoneal fluid of unknown origin in the symptomatic patient, as well as if the patient is treated with an ACEI, then the probable diagnosis is ACEI-AAE. This condition cannot be diagnosed by the current laboratory methods. The diagnostic evaluation of subcutaneous or submucosal AE unresponsive to standard therapy should always be followed by complement tests (i.e., C1-INH concentration and function/activity, C4, C1q, and anti-C1-INH autoantibody levels). One should keep in mind that ACEI medications are common triggering factors of edema formation also in patients with C1-INH deficiency; therefore, complement testing is recommended in all suspected cases of ACEI-AAE.

C1-INH deficiency can be diagnosed by the decreased C1-INH function (accompanied with low C4). Further complement tests (C1q, C1-INH concentration, anti-C1-INH antibodies) help to differentiate among the distinct forms of C1-INH deficiencies. A positive family history and/or a normal C1q level establish the diagnosis of hereditary angioedema with C1-INH deficiency (C1-INH-HAE), which has two distinct subsets based on the concentration of C1-INH. In type I, comprising 85 % of C1-INH-HAE patients, both concentration and function of C1-INH are low. In type II, however, inhibitor concentrations are normal or elevated, whereas its function is reduced (Table 1).

If C1-INH deficiency is accompanied by a negative family history and normal C1q level, decreased C1-INH function is probably caused by de novo mutation (generally the age of these patients is less than 40 years). It should be noted that ~400 different mutations in *SERPING1* gene have been demonstrated, and 25 % of diagnosed patients are considered sporadic cases, with no family history of AE [10]. If the complement profile is ambiguous, mutation analysis of the *SERPING1* gene may help. If C1-INH-HAE is suspected, complement testing should be performed in all first-degree family members, regardless of the presence or absence of symptoms. In the case of neonates born into a family where C1-INH-HAE has already been diagnosed, complement studies can be performed as early as at birth, from cord blood [11]. However, before the age of 1 year, complement levels may not reach those seen in adults, and thus, and repeated testing is recommended to confirm the initial findings after the infant turns 1 year old [12]. The results of blood C1-INH tests should be interpreted with caution in pregnant women with AE, because of the transitory decline in plasma C1-INH levels during pregnancy, consequent to the expansion of plasma volume [13]. The measurement of C1-INH levels should be repeated postpartum in order to confirm the diagnosis of C1-INH-HAE. If C1-INH deficiency is accompanied with negative family history and low C1q, then acquired angioedema with C1-INH deficiency (C1-INH-AAE) can be diagnosed [14] (generally, the age of these patients is more than 40 years). In such cases, the diagnostic steps should focus on the identification of underlying disease, such as lymphoproliferative onco-hematologic or immunoregulatory disorders, and on the detection of anti-C1-INH antibodies. Although still a debated issue, the absence of the latter identifies type I C1-INH-AAE, whereas their presence confirms type II C1-INH-AAE [15]. Although an underlying disease is less common in

Table 1 Diagnostic laboratory profile of angioedema phenotypes

Type	Disorder	fC1-INH	AgC1-INH	C4	C1q	Anti-C1-INH Abs	Mutation in *SERPING1* gene	Mutation in *FXII* gene
Acquired	IH-AAE	N	N	N	N	No	No	No
	InH-AAE	N	N	N	N	No	No	No
	ACEI-AAE	N	N	N	N	No	No	No
	C1-INH-AAE type I	↓	↓	↓	↓	No	No	No
	C1-INH-AAE type II	↓	↓/N	↓	↓	Yes	No	No
Hereditary	C1-INH-HAE type I	↓	↓	↓	N	No	Yes	No
	C1-INH-HAE type II	↓	N/↑	↓	N	No	Yes	No
	FXII-HAE	N	N	N	N	No	No	Yes
	U-HAE	N	N	N	N	No	No	No

IH-AAE idiopathic histaminergic acquired angioedema, *ACEI-AAE* acquired angioedema related to angiotensin-converting enzyme inhibitors, *C1-INH-HAE* hereditary angioedema with C1-INH deficiency, *C1-INH-AAE* acquired angioedema with C1-INH deficiency, *U-HAE* hereditary angioedema of unknown origin, *FXII-HAE* hereditary angioedema due to a mutation of the coagulation factor XII, *InH-AAE* idiopathic non-histaminergic acquired angioedema, *fC1-INH* C1-inhibitor function, *AgC1-INH* C1-inhibitor concentration (antigen), *Anti-C1-INH Ab* autoantibodies against C1-inhibitor, *N* normal level

patients with type II C1-INH-AAE, a follow-up of these patients is also justified (i.e., the presence of low-grade monoclonal proteins) because they might develop lymphoproliferative disease later in life [16, 17].

If C1-INH deficiency cannot be demonstrated in complement assays, and if it was verified that the patient does not take ACEI medications and the swelling does not resolve in response to antiallergic standard therapy, cases can be divided based on family history. If family history is negative for AE, two further types should be considered. If mutation of the FXII gene can be verified, then the diagnosis is de novo case of FXII-HAE. The tentative diagnosis of InH-AAE may be established if mutation of the FXII gene cannot be detected. In case of positive family history for AE, testing for mutations in the FXII gene may assist further classification. As previously mentioned, normal plasma C1-INH, with mutations detected in the FXII gene establishes the diagnosis of FXII-HAE (majority of patients are females). Finally, hereditary AEs without underlying C1-INH deficiency or FXII mutation should be assigned to the U-HAE category [18]. Further follow-up and evaluation to identify the underlying cause of AE are justified in cases of C1-INH-AAE, U-HAE, and InH-AAE. A suggested diagnostic protocol is outlined in Fig. 1, whereas laboratory methods for identifying the various phenotypes of AE are summarized by Table 1.

Manual of Diagnostic Laboratory Testing in Angioedema

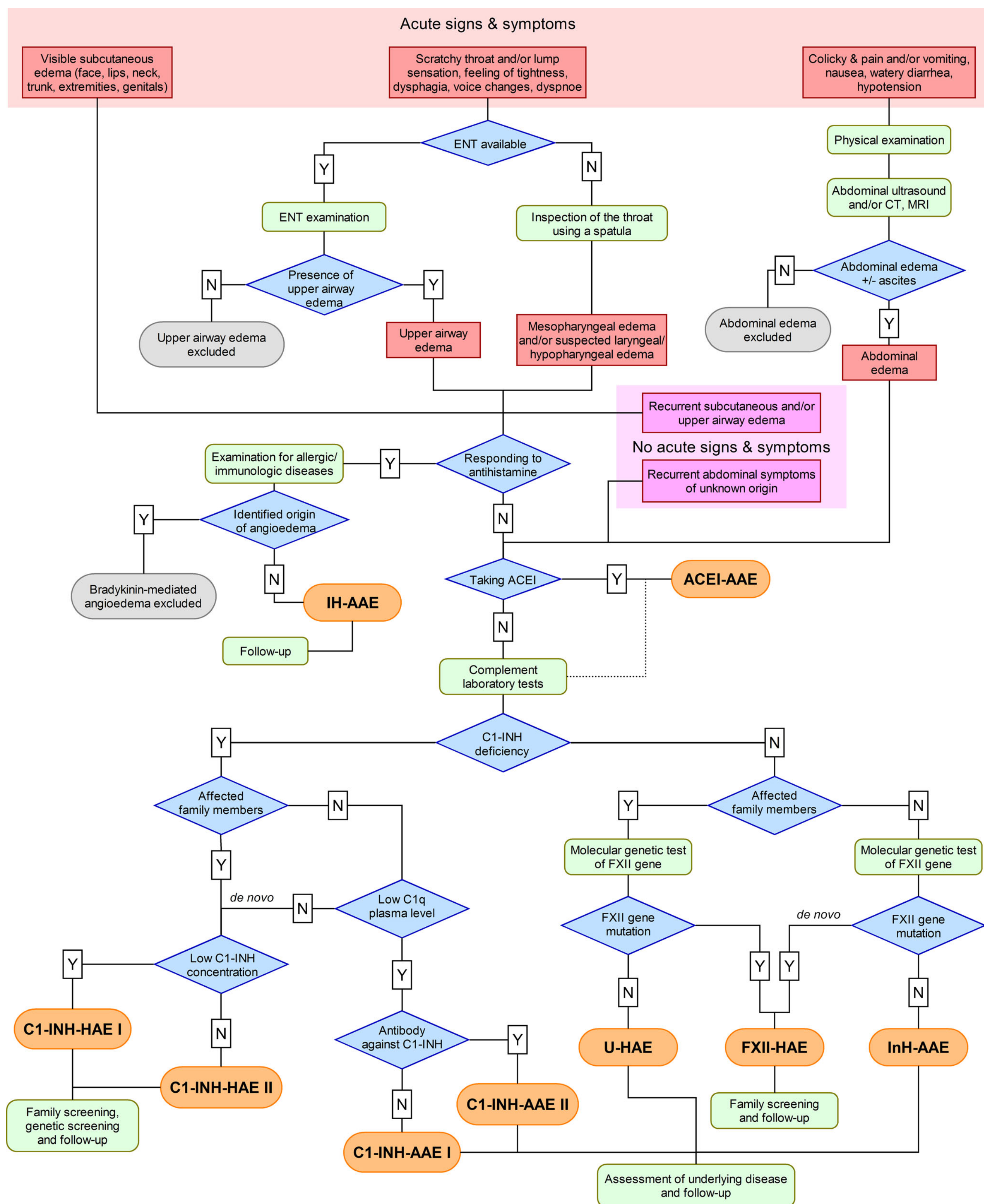
Handling of Blood Samples for Analysis

The laboratory tests appropriate for the diagnosis of AEs are usually done on blood samples. Complement tests require serum or plasma samples, whereas EDTA-anticoagulated blood should be obtained for molecular genetic studies. Inappropriate handling of the samples before analysis jeopardizes the validity of complement tests. Serum samples should be stored and transported at 20 to 25 °C during the complete coagulation process (which takes 20 min at the least and 120 min at the most), as well as during centrifugation, which should follow immediately. Cooling the samples should be avoided, because this can lead to in vitro complement activation through the classical pathway. Anticoagulated blood samples should be centrifuged as soon as possible without delay. If laboratory testing cannot be done on the day of collection, the samples should be stored in a deep freezer (−20 °C) for a short-term storage (max. 3 months) or ultra-deep freezer (−70 °C) for a long-term storage until analysis. Avoiding repeated freeze-thawing is necessary and can be achieved by storing the sample in small aliquots [19, 20]. Functional tests are particularly sensitive to changes occurring in the sample before analysis. Measurement of C1-INH function should be done in citrate-anticoagulated plasma samples, which are the most resistant to the destructive effect of storage at fluctuating temperatures [21]. Although storing blood for DNA analysis is less complicated, the quality of DNA extracted from blood samples collected for genetic studies can also be influenced by the storage conditions [22].

Diagnostic Complement Tests

Laboratory diagnostics of AEs includes the measurement of five parameters: C1-INH function, C1-INH concentration, C4, C1q, and autoantibodies against C1-INH (Table 1).

C1-INH function The methods for measuring C1-INH function are based on the fact that C1-INH is the natural, exclusive



inhibitor of the C1s protease. Using a colorimetric determination, a known quantity of active C1s enzyme is added to the sample, and its residual activity is then measured by the cleavage of an artificial colorimetric substrate. The residual activity of the C1s enzyme is inversely proportional to the function of C1-INH present in the patients' sample. The

◀ **Fig. 1** Diagnostic algorithm of angioedema. *ENT* ear-nose-throat specialist, *IH-AAE* idiopathic histaminergic acquired angioedema, *ACEI-AAE* acquired angioedema related to angiotensin-converting enzyme inhibitor, *C1-INH-HAE* hereditary angioedema with C1-INH deficiency, *C1-INH-AAE* acquired angioedema with C1-INH deficiency, *U-HAE* hereditary angioedema of unknown origin, *FXII-HAE* hereditary angioedema due to a mutation of the coagulation factor XII, *InH-AAE* idiopathic non-histaminergic acquired angioedema, *Y* yes (presence of), *N* no (absence of). The following shapes symbolize the step of the algorithm: *red rectangle*—acute signs and symptoms, *magenta rectangle*—recurrent signs and symptoms, *blue diamond*—decision mark, *green rounded rectangle*—medical or laboratory procedure, *gray oval*—excluded disease, and *orange oval*—final diagnosis

determination of function of C1-INH by ELISA is based on the stable covalent bond between active C1s and C1-INH. When added to the sample, biotin-labeled active C1s reacts with the functional C1-INH, and the quantity of the complex thus formed can be measured by ELISA. The ELISA plate is covered with avidin, which binds all the biotin-labeled C1s, regardless of its state (either complexed or free); however, only C1s/C1-INH complexes can be detected by specific anti-C1-INH antibodies. The function of C1-INH is expressed as a percentage of the normal value, but using these two types of functional tests may yield different results (>50 and >70 % are considered normal with chromogenic assay and complex-forming ELISA, respectively). The positive predictive value of the chromogenic assay is lower (the proportion of false positive determinations is greater), whereas the opposite is true for the complex-forming ELISA method (which yields more false negative results). Notwithstanding this, both methods demonstrate high sensitivity and specificity in diagnosing C1-INH deficiency, particularly when combined with C4 concentrations [21, 23, 24]. Recently, a new ELISA technique has been developed for the detection of function of C1-INH. This method measures the quantity of complexes formed by FXIIa or by kallikrein with C1-INH. These enzymes are known to have additional inhibitors, and this can interfere with detection. However, these activation products are generated also during the in vivo release of *bradykinin*, and hence, they might prove as a relevant diagnostic parameter [25].

C1-INH concentration Quantitative C1-INH serum levels are usually determined by immunochemical methods, such as nephelometry, turbidimetry, or radial immunodiffusion, using specific antibodies. C1-INH concentration is generally considered low if it is lower than 50 % of the normal value. For example, in C1-INH-HAE type I, both concentration and function of C1-INH are low, whereas in C1-INH-HAE type II, the C1-INH concentration is normal (or even elevated), but the function of the mutant C1-INH protein is greatly reduced. This discrepancy between the concentration vs. function of C1-INH may also occur in certain forms of C1-INH-AAE type II. Autoantibodies to C1-INH may functionally inactivate it, causing very low activity, but its antigenic level will be still measurable.

Complement C4 C4 concentration is also tested with the same immunochemical methods as C1-INH concentration. C4 concentration is generally considered low if it is lower than 50 % of the normal value. As a rule, C4 level is usually low in C1-INH deficiency, and being normal is an exception [26, 27]. In order to avoid misdiagnosis, it is recommended to interpret the combined results of all three parameters (function and concentration of C1-INH, as well as C4 levels).

Complement C1q 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 C1-INH deficiency. However, a transient decline of C1q level may occur as an exception also in C1-INH-HAE. This may suggest the presence of some underlying, secondary immunoregulatory disorder, and therefore, diagnostic evaluation to identify the latter is justified when C1q levels are repetitively low. On the other hand, C1q levels may be normal in certain cases of C1-INH-AAE, particularly in the autoantibody-mediated type II.

Autoantibodies against C1-INH The detection of autoantibodies against C1-INH—which are characteristic for C1-INH-AAE type II—is usually based on the binding of IgG, IgA, or IgM isotype autoantibodies to an ELISA plate covered with the C1-INH protein [16, 28]. Regarding the heterogeneity of these autoantibodies [29], completion of the measurement with their neutralizing capacity would be more relevant [30]. Only a few laboratories determine autoantibodies against C1-INH because commercial kits are not available; therefore, antibody testing is done with proprietary in-house methods. As laboratories use different sets of standard obtained from their own antibody positive patients, the results are not comparable with each other. It is our view that only consistent findings, fulfilling the classification criteria of each specific disease form, may be helpful in establishing the diagnosis. When laboratory findings are inconsistent, it is recommended to repeat the tests on fresh samples until two concordant results are obtained.

Figure 1 summarizes a suggested algorithm of diagnostic workup of the different clinical phenotypes of angioedema, based on available laboratory methods. Some requires the utilization of specialized laboratories.

Specificity vs. Sensitivity Issues

In medical diagnosis, "sensitivity" is the ability of a test to correctly identify those with the disease (true positive rate), whereas "specificity" is the ability of the test to correctly identify those without the disease (true negative rate). In the

case of AEs, it is impossible to determine these parameters of diagnostic accuracy, as means for validating the assignment of patients to the said categories (i.e., of "true patients" vs. of "true healthy individuals") are not available. In particular, the diagnostic criteria contain—along with the clinical characteristics—further indices, namely C1-INH concentration and function, as well as the values of C4 determinations. Performing genetic studies instead of complement testing is still not helpful in validating the types of hereditary HAE, because a gene mutation cannot be found in as high as 8–10 % of patients with C1 INH-HAE.

Potential Diagnostic and Disease-Activity Biomarkers of Angioedema

Apart from the precise diagnosis of AEs, intriguing questions remain open about the pathomechanisms of the disease. Moreover, we need to address clinical issues, such as metrics of disease severity, attack frequency, identification of triggering factors, prodromal signs and symptoms, and the possibility of predicting oncoming edematous attacks. To answer these important questions, disease-specific biomarkers need to be added to the above-described diagnostic panel.

According to the concise definition proposed by Strimbu and Tavel [31], any measurable parameter reflecting biological, pathological processes, or the response to therapy would be a suitable biomarker. The desired properties of a biomarker include an objective, reliable, and reproducible metrics. The contribution to at least one of the following is also necessary: diagnosis/differential diagnosis, clarification of biological mechanism/pathomechanism, choice of therapy, monitoring disease course/therapy, prediction of prognosis, or screening for the disease. Further desired parameters would be consensus-based usage and affordability even for small clinical centers. Suitable biomarkers would include nearly any medical sign from laboratory parameters through imaging findings to the results of genetic tests or "omics"; however, this review focuses only on laboratory measurements. The research in the field of AE is still young, and most of the biochemical pathways leading to its pathology were described in the last 65 years. Naturally, in a rare disease like HAE, limited sampling and the complexity of clinical situations make it difficult to assimilate even "very promising" biomarkers into clinical practice.

The most promising sources of suitable AE biomarkers appear to be the plasma enzyme cascade systems, endothelial cell-related, and inflammatory factors. Therefore, we executed a complex search based on the following: (1) PubMed (before December 8, 2015) with the term "angioedema" AND ("markers" OR "biomarkers"), (2) "hereditary angioedema" keyword in the Gobiom database (https://gobiomdb.com), and (3) several additional papers cited by the most relevant reviews [32, 33] of the field.

Table 2 presents many potential biomarkers, which have been already studied in cohorts of AE patients, and may serve to improve the diagnosis and management of AE in the future. Biomarkers have been listed according to their primary function (such as complement, contact, coagulation, and fibrinolytic systems, endothelial cell markers, neutrophil cell markers, inflammatory markers, and miscellaneous markers); however, these categories may be overlapping. We omitted data from isolated case reports along with inconsistent data, according to the biomarker definition. For the sake of clarity, we also omitted factors which were found not significantly different between healthy controls and AE patients or between samples obtained from the patients during symptom-free or during attack periods. In our review of AE biomarkers, we found inconsistent data even regarding the notable factors involved in the pathogenesis of AEs (e.g., bradykinin levels measured in the patients during symptom-free periods vs. those of healthy controls [47, 72, 73], FXII activity in controls and in patients during symptom-free periods [74, 75]). Controversial results have been published regarding the relevance of PAI-1 levels [41, 54]. Although these have been omitted from the Table 2, several remarkable, non-laboratory, and molecular genetic biomarker candidates deserve mentioning. It was suggested that gender, race, age, and certain comorbidities (e.g., diabetes mellitus, allergy, and posttransplant state) can influence the onset of the symptoms and/or the frequency or the severity of edematous attacks [46, 52, 76–78]. Several polymorphisms/mutations of possibly AE-related genes might also prove good biomarkers, including the bradykinin receptor B2R [79, 80], FXII [81, 82], MBL2 [38], neprilysin [83], and aminopeptidase P [49].

Other Biologic Parameters

Besides genetic and non-laboratory parameters, other biological metrics can be utilized. One example is the coronary flow reserve (measured by Doppler ultrasound), which was shown to be lower in C1-INH-HAE patients than in healthy controls [84]. Several other interesting factors have been described in the literature. Although at the present time these do not fulfill the criteria for biomarkers because of the low sample size (case reports) or of the purpose of the study (investigation of the in vitro pathogenetic background), these studies may have "stumbled" upon new, possible biomarkers. These may include cytokines (IL-1beta, IL-4, IL-5, IL-6, IL-13 [69, 85]), elements of the plasma cascade systems (soluble thrombomodulin, thrombin activatable fibrinolysis inhibitor [56]), plasma kininogenase activity [86]), soluble form of endothelial cell adhesion molecules (P-selectin [87], VE-cadherin [86]), tumor markers (CA 125 [88]), and extracellular pool of stress proteins (Hsp90 [89]).

Table 2 Potential biomarkers of angioedema

Biomarker	Biologic function	Angioedema types				Relevance[a]
		C1-INH-HAE	FXII-HAE	C1-INH-AAE	ACEI-AAE	
C1-inhibitor quantity (antigenic, AgC1-INH)	Serin protease inhibitor of the complement, contact/kinin, coagulation, and fibrinolytic systems	x				Serum level under 0.075 g/l predisposes to edematous attacks [34]
C1-inhibitor function (fC1-INH)	Idem	x				Correlates with disease severity [35, 36]; may predict attack frequency and C1-INH concentrate consumption [36]
C4	Part of a C3-convertase complex of the classic complement pathway	x				Correlates with attack frequency and C1-INH concentrate consumption in patients treated with danazol [36]
C1/C1-INH complex	Stable complex of inactivated C1s/r (esterase) with C1-INH	x				Higher[b] in HAE patients; may predict disease severity [36], and increases further[c] during attacks [37]
Classic complement pathway activity	Immunoglobulin/CRP induced activation of complement system	x				Lower activity in HAE patients [38]
C3	Central component of complement system	x				Lower level in some HAE patients [38]
MASP-2	Serine protease of the lectin pathway	x				Lower level in HAE [38]; however, increases during attacks [39]
MASP-1	Serine protease of the lectin pathway	x				Lower level in patients; negative correlation with attack frequency [40]
MASP-1/C1-INH complex	Stable complex of inactivated MASP-1 with C1-INH	x				Lower level in patients; negative correlation with attack frequency [40]
MASP-1/antithrombin (AT) complex	Stable complex of inactivated MASP-1 with AT	x				Lower level in patients [40]
MASP-2/ficolin-3 complex	Stable complex of inactivated MASP-2 with ficolin-3	x				Lower level in patients; however, increases during attacks compared with inter-attack periods [39]
Ficolin-3	Recognition molecule of lectin pathway	x				Lower deposition of ficolin-3 mediated terminal complement complex in patients decreases further during attacks [39]
Anti-C1-INH IgM antibody	Autoantibodies against C1-INH	x		x		Correlation with disease activity in C1-INH-HAE patients naive to treatment with C1-INH concentrate [28]; higher level in C1-INH-AAE patients; pathogenetic role of the disease [29]
Anti-C1-INH IgG and IgA antibodies	Autoantibodies against C1-INH			x		Higher level in patients; pathogenetic role in the disease [29]
Activated factor XII (FXIIa)	Serine protease of the contact system	x		x		Higher level in HAE patients [41, 42]; increases further during C1-INH-HAE and C1-INH-AAE attacks [41–43]
Factor XIIa/C1-INH complex	Stable complex of inactivated FXIIa with C1-INH	x				Lower level in patients [44]
Kallikrein	Serine protease of the contact system	x				Higher activity in patients, increases further during attacks [42, 44, 45]
Cleaved high-molecular-weight kininogen (HK)	Proteolytic product of HK when kallikrein release BK	x				Higher level in patients, increases further during attacks [45]
Kallikrein/C1-INH complex	Stable complex of inactivated kallikrein with C1-INH	x				Lower level in patients [44]
Bradykinin (BK)	Vasoactive peptide, key mediator of AEs	x			x	Increased level during attacks [46, 47]
Plasma amidase activity	Total activity of serine proteases involved in BK formation	x	x	x		Higher in C1-INH-HAE, FXII-HAE and C1-INH-AAE; further increase in FXII-HAE during attacks [48]
Aminopeptidase P (APP)	BK cleaving cell surface peptidase	x			x	Lower level in ACEI-AAE patients [49]; activity inversely correlates

Table 2 (continued)

Biomarker	Biologic function	Angioedema types				Relevance[a]
		C1-INH-HAE	FXII-HAE	C1-INH-AAE	ACEI-AAE	
						with disease severity in C1-INH-HAE [50]
Carboxy-peptidase N (CPN)	BK cleaving cell surface peptidase	x				Inversely correlates with disease severity in patients not treated with danazol [50]
Dipeptidyl-peptidase IV (DPPIV)	BK cleaving cell surface peptidase				x	Lower level in ACEI-AAE compared with ACEI without angioedema; predictor of attacks [51–53]
Alpha-2-macroglobulin	Plasma serine protease inhibitor		x			Lower level in patients [54]
Factor XIa (FXIa)	Serine protease of the coagulation system	x				Higher level in patients [41]
FXIa/C1-INH complex	Stable complex of inactivated FXIa with C1-INH	x				Lower level in patients [44]
Factor VIIa	Serine protease of the coagulation system	x		x		Increased level during attacks [55]
Thrombin	Serine protease of the coagulation system (a strong vasoactive agent)	x				Higher activity in patients; increases further during attacks, when the plasma is activated in vitro [56]
Activated partial thromboplastin time (aPTT)	Reflects to the intrinsic pathway activity	x				Shorter in patients; decreases further during attacks [41]
Prothrombin time (PT)	Reflects to the extrinsic pathway activity	x				Shorter during attacks [41]
Prothrombin fragments 1 + 2	Generated during thrombin activation	x				Higher level in patients; increases further during attacks [41, 56, 57]
Thrombin/antithrombin complex	Stable complex of inactivated thrombin with AT	x				Higher level in patients [41]; increases further during attacks [41, 58]
Fibrinogen	Pro-form of the clot forming fibrin	x			x	Increased level during attacks [41, 59]
Plasminogen activator inhibitor-1 (PAI-1)	Inhibitor of plasminogen activator	x				decreased level during attacks [41]
Plasminogen activator inhibitor-2 (PAI-2)	Inhibitor of plasminogen activator	x	x			Lower in FXII-HAE patients, higher in C1-INH-HAE patients [54]
Plasmin/antiplasmin (AP) complex	Stable complex of inactivated plasmin with AP	x				Higher level in patients, increases further during attacks [41, 42, 56, 60]
D-dimer	Lytic fragment of fibrin degradation (following fXIII stabilization)	x				Higher level in patients; increases further during attacks [41, 56, 61]; higher level in abdominal AE than in non-AE abdominal attacks [57]
Soluble E-selectin	Carbohydrate binding adhesion molecule of endothelial cells	x				Higher level in patients, further increased during attacks [62, 63]
von Willebrand factor (vWF)	Endothelial cell and platelet derived coagulation factor	x				Increased level and collagen binding activity during attacks [63]
Endothelin-1	Vasoactive peptide produced by endothelial cells	x				Increased level during attacks [63, 64]
Adrenomedullin	Vasoactive peptide produced by endothelial cells	x				Increased level during attacks [64]
Arginine vasopressin	Vasoactive peptide	x				Increased level during attacks [64]
Atrial natriuretic peptide	Vasoactive peptide	x				Lower level in patients [65]
Vascular endothelial growth factor A and C (VEGF)	Growth and permeability factor for endothelial cells	x				Higher level in patients [66]
Angiopoietin 1 and 2	Angiogenetic factor	x				Higher level in patients [66]
C-reactive protein (CRP)	Pattern recognition acute phase protein	x			x	Increased level during attacks [59, 67, 68]
Pentraxin 3	Pattern recognition acute phase protein	x				Increased level during attacks [68]
Erythrocyte sedimentation rate (ESR)	Reflects active inflammation	x				Increased level during attacks [67]
White blood cell count (WBC)	Reflects overall inflammation and stress	x				Higher level in patients; increases further during attacks [68]
Neutrophil count	Reflects special inflammatory processes and stress	x				Higher level in patients; increases further during attacks [68]

Table 2 (continued)

Biomarker	Biologic function	Angioedema types				Relevance[a]
		C1-INH-HAE	FXII-HAE	C1-INH-AAE	ACEI-AAE	
Neutrophil elastase	Extracellular matrix cleaving serine protease of neutrophils	x				Increased level during attacks [68]
Myeloperoxidase	antibacterial enzyme from neutrophils	x				Increased level during attacks [68]
Tumor necrosis factor alpha (TNF)	Pro-inflammatory cytokine	x				Increased level during attacks [68, 69]
Granulocyte colony stimulating factor (G-CSF)	Growth factor for leukocyte subpopulations	x				Higher level in patients [70]
Granulocyte-macrophage colony stimulating factor (GM-CSF)	Growth factor for leukocyte subpopulations	x				Higher level in patients; increases further during attacks [70]
Interleukin-8 (IL-8)	Chemotactic cytokine for neutrophils	x				Increased level during attacks [68]
Interleukin-17 (IL-17)	Proinflammatory cytokine	x				Higher level in patients; increases further during attacks [69, 70]
Basic fibroblast growth factor	Growth factor for fibroblasts, smooth muscle cells and endothelial cells	x				Higher level in patients; increases further during attacks [70]
Progesterone	Sex hormone	x				Its level positively correlates with attack frequency [71]
Sex hormone binding globulin (SHBG)	Associates with steroid hormones in the blood plasma	x				Its level negatively correlates with attack frequency [71]

[a] Due to space limitations, this table is oversimplified, i.e., in some instances, our statements pertain to a subgroup of the cohort

[b] Unless stated otherwise, "higher" or "lower" always refer to the patients compared with the healthy controls

[c] Unless stated otherwise, "increased" or "decreased" always refer to samples obtained during an attack compared with samples from symptom-free patients

Future Perspectives

Obtaining more data to solve the still unrevealed fields of AEs is highly desirable.

- First, as required by the definition of a "good biomarker," the reproducibility of the potential biomarkers shown in Table 2 should be verified in larger cohort of patients. This should be done by conducting large-scale, international, multicenter studies, enrolling as many patients (and controls) as possible.
- Study methods should be based on those adopted by well-designed studies and should include appropriate handling of samples, as well as standardized assays and pertinent statistical analyses. Examples for key parameters include self-controlled study design for the evaluation of AE attacks, minimization of time between the collection and freezing of blood samples, and preferential utilization of tests involved in external quality assessment.
- Additionally, combinations of several biomarkers, when analyzed by robust statistical methods (e.g., logical analysis of data), may better identify disease-related conditions. This may lead to a more accurate diagnosis in real time, as well as techniques with reliable power to predict of the onset of edematous attacks and disease course.
- Eventually, by using appropriate laboratory measurements, therapeutic regimens can be improved to better suit the individual needs of the patients.

Conclusions

The classification, laboratory diagnostics as well as the treatment of AEs have substantially progressed in the last decade. However, the "nuts and bolts" of laboratory diagnosis are currently not applicable for all types of AEs, since only five of the nine types of AEs can be identified by laboratory methods (i.e., complement tests and molecular genetic methods). Moreover, the definitive diagnosis of these five types of AEs requires several days, and this is unacceptable in the case of a potentially life-threatening disease. Clinicians should take into account the clinical signs and symptoms, family history, therapeutic response, and the diagnostic laboratory profile together to accelerate and improve the diagnostic process. The progressive utilization of novel biomarkers

might lead to more specific diagnoses, better management, and individualized therapy.

Acknowledgments The study was supported by the grant of the Hungarian Scientific Research Fund (OTKA) 100886 (HF). All authors have equally contributed to this work.

References

1. Cicardi M, Aberer W, Banerji A, Bas M, Bernstein JA, Bork K, Caballero T, Farkas H, Grumach A, Kaplan AP, Riedl MA, Triggiani M, Zanichelli A, Zuraw B (2014) Classification, diagnosis, and approach to treatment for angioedema: consensus report from the Hereditary Angioedema International Working Group. Allergy 69(5):602–616. doi:10.1111/all.12380
2. Wu MA, Perego F, Zanichelli A, Cicardi M (2016) Angioedema phenotypes: disease expression and classification. Clinic Rev Allerg Immunol. doi:10.1007/s12016-016-8541-z
3. Farkas H (2010) Management of upper airway edema caused by hereditary angioedema. Allergy Asthma Clin Immunol 6(1):19. doi:10.1186/1710-1492-6-19
4. Bork K, Meng G, Staubach P, Hardt J (2006) Hereditary angioedema: new findings concerning symptoms, affected organs, and course. Am J Med 119(3):267–274. doi:10.1016/j.amjmed.2005.09.064
5. Farkas H, Harmat G, Kaposi PN, Karadi I, Fekete B, Fust G, Fay K, Vass A, Varga L (2001) Ultrasonography in the diagnosis and monitoring of ascites in acute abdominal attacks of hereditary angioneurotic oedema. Eur J Gastroenterol Hepatol 13(10):1225–1230
6. Ali MA, Borum ML (2014) Hereditary angioedema: what the gastroenterologist needs to know. Clin Exp Gastroenterol 7:435–445. doi:10.2147/CEG.S50465
7. Zingale LC, Zanichelli A, Deliliers DL, Rondonotti E, De Franchis R, Cicardi M (2008) Successful resolution of bowel obstruction in a patient with hereditary angioedema. Eur J Gastroenterol Hepatol 20(6):583–587. doi:10.1097/MEG.0b013e3282f1c995
8. Farkas H, Csuka D (2013) An abdominal attack of hereditary angiooedema. Lancet 381(9875):1404. doi:10.1016/S0140-6736(12)61146-5
9. Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW, Church MK, Ensina LF, Gimenez-Arnau A, Godse K, Goncalo M, Grattan C, Hebert J, Hide M, Kaplan A, Kapp A, Abdul Latiff AH, Mathelier-Fusade P, Metz M, Nast A, Saini SS, Sanchez-Borges M, Schmid-Grendelmeier P, Simons FE, Staubach P, Sussman G, Toubi E, Vena GA, Wedi B, Zhu XJ, Maurer M (2014) The EAACI/GA(2) LEN/EDF/WAO guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. Allergy 69(7):868–887. doi:10.1111/all.12313
10. Germenis AE, Speletas M (2016) Genetics of hereditary angioedema revisited. Clinic Rev Allerg Immunol. doi:10.1007/s12016-016-8543-x
11. Nielsen EW, Johansen HT, Holt J, Mollnes TE (1994) C1 inhibitor and diagnosis of hereditary angioedema in newborns. Pediatr Res 35(2):184–187. doi:10.1203/00006450-199402000-00012
12. Pedrosa M, Boyano-Martinez T, Garcia-Ara C, Caballero T, Quirce S (2015) Utility of specific IgE to Ara h 6 in peanut allergy diagnosis. Ann Allergy Asthma Immunol 115(2):108–112. doi:10.1016/j.anai.2015.05.015
13. Walker JE, Campbell DM, Ogston D (1982) Blood levels of proteinase inhibitors in pregnancy. Br J Obstet Gynaecol 89(3):208–210
14. Oltvai ZN, Wong EC, Atkinson JP, Tung KS (1991) C1 inhibitor deficiency: molecular and immunologic basis of hereditary and acquired angioedema. Lab Invest 65(4):381–388
15. Alsenz J, Bork K, Loos M (1987) Autoantibody-mediated acquired deficiency of C1 inhibitor. N Engl J Med 316(22):1360–1366. doi:10.1056/NEJM198705283162202
16. Cicardi M, Beretta A, Colombo M, Gioffre D, Cugno M, Agostoni A (1996) Relevance of lymphoproliferative disorders and of anti-C1 inhibitor autoantibodies in acquired angio-oedema. Clin Exp Immunol 106(3):475–480
17. Cicardi M, Zingale LC, Pappalardo E, Folcioni A, Agostoni A (2003) Autoantibodies and lymphoproliferative diseases in acquired C1-inhibitor deficiencies. Medicine 82(4):274–281. doi:10.1097/01.md.0000085055.63483.09
18. Bork K, Wulff K, Witzke G, Hardt J (2015) Hereditary angioedema with normal C1-INH with versus without specific F12 gene mutations. Allergy 70(8):1004–1012. doi:10.1111/all.12648
19. Frazer-Abel A, Giclas PC (2011) Update on laboratory tests for the diagnosis and differentiation of hereditary angioedema and acquired angioedema. Allergy Asthma Proc 32(Suppl 1):S17–21. doi:10.2500/aap.2011.32.3472
20. Lachmann PJ (2010) Preparing serum for functional complement assays. J Immunol Methods 352(1–2):195–197. doi:10.1016/j.jim.2009.11.003
21. Wagenaar-Bos IG, Drouet C, Aygoren-Pursun E, Bork K, Bucher C, Bygum A, Farkas H, Fust G, Gregorek H, Hack CE, Hickey A, Joller-Jemelka HI, Kapusta M, Kreuz W, Longhurst H, Lopez-Trascasa M, Madalinski K, Naskalski J, Nieuwenhuys E, Ponard D, Truedsson L, Varga L, Nielsen EW, Wagner E, Zingale L, Cicardi M, van Ham SM (2008) Functional C1-inhibitor diagnostics in hereditary angioedema: assay evaluation and recommendations. J Immunol Methods 338(1–2):14–20. doi:10.1016/j.jim.2008.06.004
22. Malentacchi F, Pizzamiglio S, Ibrahim-Gawel H, Pazzagli M, Verderio P, Ciniselli CM, Wyrich R, Gelmini S (2015) Second SPIDIA-DNA External Quality Assessment (EQA): influence of pre-analytical phase of blood samples on genomic DNA quality. Clin Chim Acta. doi:10.1016/j.cca.2015.12.032
23. Li HH, Busse P, Lumry WR, Frazer-Abel A, Levy H, Steele T, Dayno J, Riedl M (2015) Comparison of chromogenic and ELISA functional C1 inhibitor tests in diagnosing hereditary angioedema. J Allergy Clin Immunol In Pract 3(2):200–205. doi:10.1016/j.jaip.2014.08.002
24. Gompels MM, Lock RJ, Morgan JE, Osborne J, Brown A, Virgo PF (2002) A multicentre evaluation of the diagnostic efficiency of serological investigations for C1 inhibitor deficiency. J Clin Pathol 55(2):145–147
25. Joseph K, Bains S, Tholanikunnel BG, Bygum A, Aabom A, Koch C, Farkas H, Varga L, Ghebrehiwet B, Kaplan AP (2015) A novel assay to diagnose hereditary angioedema utilizing inhibition of bradykinin-forming enzymes. Allergy 70(1):115–119. doi:10.1111/all.12520
26. Tarzi MD, Hickey A, Forster T, Mohammadi M, Longhurst HJ (2007) An evaluation of tests used for the diagnosis and monitoring of C1 inhibitor deficiency: normal serum C4 does not exclude hereditary angio-oedema. Clin Exp Immunol 149(3):513–516. doi:10.1111/j.1365-2249.2007.03438.x
27. Gompels MM, Lock RJ, Unsworth DJ, Johnston SL, Archer CB, Davies SV (2003) Misdiagnosis of hereditary angio-oedema type 1 and type 2. Br J Dermatol 148(4):719–723
28. Varga L, Szeplaki G, Visy B, Fust G, Harmat G, Miklos K, Nemeth J, Cervenak L, Karadi I, Farkas H (2007) C1-inhibitor (C1-INH) autoantibodies in hereditary angioedema. Strong correlation with the severity of disease in C1-INH concentrate naive patients. Mol Immunol 44(6):1454–1460. doi:10.1016/j.molimm.2006.04.020

29. Cicardi M, Bergamaschini L, Cugno M, Beretta A, Zingale LC, Colombo M, Agostoni A (1998) Pathogenetic and clinical aspects of C1 inhibitor deficiency. Immunobiology 199(2):366–376. doi:10.1016/S0171-2985(98)80041-7
30. Engel R, Rensink I, Roem D, Brouwer M, Kalei A, Perry D, Zeerleder S, Wouters D, Hamann D (2015) ELISA to measure neutralizing capacity of anti-C1-inhibitor antibodies in plasma of angioedema patients. J Immunol Methods 426:114–119. doi:10.1016/j.jim.2015.08.011
31. Strimbu K, Tavel JA (2010) What are biomarkers? Curr Opin HIV AIDS 5(6):463–466. doi:10.1097/COH.0b013e32833ed177
32. Deroux A, Vilgrain I, Dumestre-Perard C, Boccon-Gibod I, Bouillet L (2015) Towards a specific marker for acute bradykinin-mediated angioedema attacks: a literature review. Eur J Dermatol EJD 25(4):290–295. doi:10.1684/ejd.2015.2547
33. Kaplan AP, Joseph K (2010) The bradykinin-forming cascade and its role in hereditary angioedema. Ann Allergy Asthma Immunol 104(3):193–204. doi:10.1016/j.anai.2010.01.007
34. Spath PJ, Wuthrich B, Butler R (1984) Quantification of C1-inhibitor functional activities by immunodiffusion assay in plasma of patients with hereditary angioedema—evidence of a functionally critical level of C1-inhibitor concentration. Complement 1(3):147–159
35. Kelemen Z, Moldovan D, Mihaly E, Visy B, Szeplaki G, Csuka D, Fust G, Farkas H, Varga L (2010) Baseline level of functional C1-inhibitor correlates with disease severity scores in hereditary angioedema. Clin Immunol 134(3):354–358. doi:10.1016/j.clim.2009.11.002
36. Csuka D, Fust G, Farkas H, Varga L (2011) Parameters of the classical complement pathway predict disease severity in hereditary angioedema. Clin Immunol 139(1):85–93. doi:10.1016/j.clim.2011.01.003
37. Cugno M, Hack CE, de Boer JP, Eerenberg AJ, Agostoni A, Cicardi M (1993) Generation of plasmin during acute attacks of hereditary angioedema. J Lab Clin Med 121(1):38–43
38. Varga L, Szeplaki G, Laki J, Kocsis A, Kristof K, Gal P, Bajtay Z, Wieslander J, Daha MR, Garred P, Madsen HO, Fust G, Farkas H (2008) Depressed activation of the lectin pathway of complement in hereditary angioedema. Clin Exp Immunol 153(1):68–74. doi:10.1111/j.1365-2249.2008.03671.x
39. Csuka D, Munthe-Fog L, Hein E, Zotter Z, Prohaszka Z, Farkas H, Varga L, Garred P (2014) Activation of the ficolin-lectin pathway during attacks of hereditary angioedema. J Allergy Clin Immunol 134(6):1388–1393 e1381. doi:10.1016/j.jaci.2014.05.030
40. Hansen CB, Csuka D, Munthe-Fog L, Varga L, Farkas H, Hansen KM, Koch C, Skjodt K, Garred P, Skjoedt MO (2015) The levels of the lectin pathway serine protease MASP-1 and its complex formation with C1 inhibitor are linked to the severity of hereditary angioedema. J Immunol 195(8):3596–3604. doi:10.4049/jimmunol.1402838
41. Csuka D, Veszeli N, Imreh E, Zotter Z, Skopal J, Prohaszka Z, Varga L, Farkas H (2015) Comprehensive study into the activation of the plasma enzyme systems during attacks of hereditary angioedema due to C1-inhibitor deficiency. Orphanet J Rare Dis 10:132. doi:10.1186/s13023-015-0351-5
42. Joseph K, Tuscano TB, Kaplan AP (2008) Studies of the mechanisms of bradykinin generation in hereditary angioedema plasma. Ann Allergy Asthma Immunol 101(3):279–286. doi:10.1016/S1081-1206(10)60493-0
43. Cugno M, Cicardi M, Coppola R, Agostoni A (1996) Activation of factor XII and cleavage of high molecular weight kininogen during acute attacks in hereditary and acquired C1-inhibitor deficiencies. Immunopharmacology 33(1–3):361–364
44. Konings J, Cugno M, Suffritti C, Ten Cate H, Cicardi M, Govers-Riemslag JW (2013) Ongoing contact activation in patients with hereditary angioedema. PLoS One 8(8):e74043. doi:10.1371/journal.pone.0074043
45. Suffritti C, Zanichelli A, Maggioni L, Bonanni E, Cugno M, Cicardi M (2014) High-molecular-weight kininogen cleavage correlates with disease states in the bradykinin-mediated angioedema due to hereditary C1-inhibitor deficiency. Clin Exp Allergy 44(12):1503–1514. doi:10.1111/cea.12293
46. Hoover T, Lippmann M, Grouzmann E, Marceau F, Herscu P (2010) Angiotensin converting enzyme inhibitor induced angio-oedema: a review of the pathophysiology and risk factors. Clin Exp Allergy 40(1):50–61. doi:10.1111/j.1365-2222.2009.03323.x
47. Nussberger J, Cugno M, Amstutz C, Cicardi M, Pellacani A, Agostoni A (1998) Plasma bradykinin in angio-oedema. Lancet 351(9117):1693–1697. doi:10.1016/S0140-6736(97)09137-X
48. Defendi F, Charignon D, Ghannam A, Baroso R, Csopaki F, Allegret-Cadet M, Ponard D, Favier B, Cichon S, Nicolie B, Fain O, Martin L, Drouet C, National Reference Centre for Angioedema C (2013) Enzymatic assays for the diagnosis of bradykinin-dependent angioedema. PLoS One 8(8), e70140. doi:10.1371/journal.pone.0070140
49. Duan QL, Nikpoor B, Dube MP, Molinaro G, Meijer IA, Dion P, Rochefort D, Saint-Onge J, Flury L, Brown NJ, Gainer JV, Rouleau JL, Agostoni A, Cugno M, Simon P, Clavel P, Potier J, Wehbe B, Benarbia S, Marc-Aurele J, Chanard J, Foroud T, Adam A, Rouleau GA (2005) A variant in XPNPEP2 is associated with angioedema induced by angiotensin I-converting enzyme inhibitors. Am J Hum Genet 77(4):617–626. doi:10.1086/496899
50. Drouet C, Desormeaux A, Robillard J, Ponard D, Bouillet L, Martin L, Kanny G, Moneret-Vautrin DA, Bosson JL, Quesada JL, Lopez-Trascasa M, Adam A (2008) Metallopeptidase activities in hereditary angioedema: effect of androgen prophylaxis on plasma aminopeptidase P. J Allergy Clin Immunol 121(2):429–433. doi:10.1016/j.jaci.2007.10.048
51. Byrd JB, Touzin K, Sile S, Gainer JV, Yu C, Nadeau J, Adam A, Brown NJ (2008) Dipeptidyl peptidase IV in angiotensin-converting enzyme inhibitor associated angioedema. Hypertension 51(1):141–147. doi:10.1161/HYPERTENSIONAHA.107.096552
52. Grouzmann E, Livio F, Buclin T (2009) Angiotensin-converting enzyme and dipeptidyl peptidase IV inhibitors: an increased risk of angioedema. Hypertension 54(3):468–470. doi:10.1161/HYPERTENSIONAHA.109.135244
53. Lefebvre J, Murphey LJ, Hartert TV, Jiao Shan R, Simmons WH, Brown NJ (2002) Dipeptidyl peptidase IV activity in patients with ACE-inhibitor-associated angioedema. Hypertension 39(2 Pt 2):460–464
54. Joseph K, Tholanikunnel BG, Wolf B, Bork K, Kaplan AP (2015) Deficiency of plasminogen activator inhibitor 2 in plasma of patients with hereditary angioedema with normal C1 inhibitor levels. J Allergy Clin Immunol. doi:10.1016/j.jaci.2015.07.041
55. Cugno M, Cicardi M, Bottasso B, Coppola R, Paonessa R, Mannucci PM, Agostoni A (1997) Activation of the coagulation cascade in C1-inhibitor deficiencies. Blood 89(9):3213–3218
56. van Geffen M, Cugno M, Lap P, Loof A, Cicardi M, van Heerde W (2012) Alterations of coagulation and fibrinolysis in patients with angioedema due to C1-inhibitor deficiency. Clin Exp Immunol 167(3):472–478. doi:10.1111/j.1365-2249.2011.04541.x
57. Cugno M, Zanichelli A, Bellatorre AG, Griffini S, Cicardi M (2009) Plasma biomarkers of acute attacks in patients with angioedema due to C1-inhibitor deficiency. Allergy 64(2):254–257. doi:10.1111/j.1398-9995.2008.01859.x
58. Nielsen EW, Johansen HT, Hogasen K, Wuillemin W, Hack CE, Mollnes TE (1996) Activation of the complement, coagulation, fibrinolytic and kallikrein-kinin systems during attacks of hereditary angioedema. Scand J Immunol 44(2):185–192
59. Bas M, Hoffmann TK, Bier H, Kojda G (2005) Increased C-reactive protein in ACE-inhibitor-induced angioedema. Br J Clin Pharmacol 59(2):233–238. doi:10.1111/j.1365-2125.2004.02268.x

60. Joseph K, Tholanikunnel TE, Kaplan AP (2010) Treatment of episodes of hereditary angioedema with C1 inhibitor: serial assessment of observed abnormalities of the plasma bradykinin-forming pathway and fibrinolysis. Ann Allergy Asthma Immunol 104(1): 50–54. doi:10.1016/j.anai.2009.11.014
61. Reshef A, Zanichelli A, Longhurst H, Relan A, Hack CE (2015) Elevated D-dimers in attacks of hereditary angioedema are not associated with increased thrombotic risk. Allergy 70(5):506–513. doi:10.1111/all.12587
62. Czucz J, Schaffer G, Csuka D, Walentin S, Kunde J, Prohaszka Z, Farkas H, Cervenak L (2012) Endothelial cell function in patients with hereditary angioedema: elevated soluble E-selectin level during inter-attack periods. J Clin Immunol 32(1):61–69. doi:10.1007/s10875-011-9606-7
63. Kajdacsi E, Jani PK, Csuka D, Varga LA, Prohaszka Z, Farkas H, Cervenak L (2014) Endothelial cell activation during edematous attacks of hereditary angioedema types I and II. J Allergy Clin Immunol 133(6):1686–1691. doi:10.1016/j.jaci.2013.12.1072
64. Kajdacsi E, Jani PK, Csuka D, Varga L, Prohaszka Z, Farkas H, Cervenak L (2016) Novel vasoregulatory aspects of hereditary angioedema: the role of arginine vasopressin, adrenomedullin and endothelin-1. J Clin Immunol. doi:10.1007/s10875-016-0239-8
65. Kajdacsi E, Varga L, Prohaszka Z, Farkas H, Cervenak L (2016) Atrial natriuretic peptide as a novel biomarker of hereditary angioedema. Clin Immunol. doi:10.1016/j.clim.2016.03.007
66. Loffredo S, Bova M, Suffritti C, Borriello F, Zanichelli A, Petraroli A, Varricchi G, Triggiani M, Cicardi M, Marone G (2016) Elevated plasma levels of vascular permeability factors in C1 inhibitor-deficient hereditary angioedema. Allergy. doi:10.1111/all.12862
67. Hofman ZL, Relan A, Hack CE (2014) C-reactive protein levels in hereditary angioedema. Clin Exp Immunol 177(1):280–286. doi:10.1111/cei.12314
68. Veszeli N, Csuka D, Zotter Z, Imreh E, Jozsi M, Benedek S, Varga L, Farkas H (2015) Neutrophil activation during attacks in patients with hereditary angioedema due to C1-inhibitor deficiency. Orphanet J Rare Dis 10(1):156. doi:10.1186/s13023-015-0374-y
69. Arcoleo F, Salemi M, La Porta A, Selvaggio V, Mandala V, Muggeo V, Misiano G, Milano S, Romano GC, Cillari E (2014) Upregulation of cytokines and IL-17 in patients with hereditary angioedema. Clin Chem Lab Med 52(5):e91–93. doi:10.1515/cclm-2013-1008
70. Salemi M, Mandala V, Muggeo V, Misiano G, Milano S, Colonna-Romano G, Arcoleo F, Cillari E (2015) Growth factors and IL-17 in hereditary angioedema. Clin Exp Med. doi:10.1007/s10238-015-0340-y
71. Visy B, Fust G, Varga L, Szendei G, Takacs E, Karadi I, Fekete B, Harmat G, Farkas H (2004) Sex hormones in hereditary angioneurotic oedema. Clin Endocrinol 60(4):508–515. doi:10.1111/j.1365-2265.2004.02009.x
72. Shoemaker LR, Schurman SJ, Donaldson VH, Davis AE 3rd (1994) Hereditary angioneurotic oedema: characterization of plasma kinin and vascular permeability-enhancing activities. Clin Exp Immunol 95(1):22–28
73. Cugno M, Nussberger J, Cicardi M, Agostoni A (2003) Bradykinin and the pathophysiology of angioedema. Int Immunopharmacol 3(3):311–317. doi:10.1016/S1567-5769(02)00162-5
74. Cichon S, Martin L, Hennies HC, Muller F, Van Driessche K, Karpushova A, Stevens W, Colombo R, Renne T, Drouet C, Bork K, Nothen MM (2006) Increased activity of coagulation factor XII (Hageman factor) causes hereditary angioedema type III. Am J Hum Genet 79(6):1098–1104. doi:10.1086/509899
75. Bork K, Wulff K, Hardt J, Witzke G, Staubach P (2009) Hereditary angioedema caused by missense mutations in the factor XII gene: clinical features, trigger factors, and therapy. J Allergy Clin Immunol 124(1):129–134. doi:10.1016/j.jaci.2009.03.038
76. Byrd JB, Woodard-Grice A, Stone E, Lucisano A, Schaefer H, Yu C, Eyler AE, Salloum NE, Brown NJ (2010) Association of angiotensin-converting enzyme inhibitor-associated angioedema with transplant and immunosuppressant use. Allergy 65(11): 1381–1387. doi:10.1111/j.1398-9995.2010.02398.x
77. Materson BJ, Bernal EM (2009) Inherent inaccuracies and potential utility of race/ethnicity labeling in the treatment of hypertension. J Am Soc Hypertens JASH 3(5):291–294. doi:10.1016/j.jash.2009.08.001
78. Grouzmann E, Buclin T (2008) Is dipeptidylpeptidase IV the missing link in angiotensin-converting enzyme inhibitor—induced angioedema? Hypertension 51(1):45–47. doi:10.1161/HYPERTENSIONAHA.107.101329
79. Moreau ME, Garbacki N, Molinaro G, Brown NJ, Marceau F, Adam A (2005) The kallikrein-kinin system: current and future pharmacological targets. J Pharmacol Sci 99(1):6–38
80. Moholisa RR, Rayner BR, Patricia Owen E, Schwager SL, Stark JS, Badri M, Cupido CL, Sturrock ED (2013) Association of B2 receptor polymorphisms and ACE activity with ACE inhibitor-induced angioedema in black and mixed-race South Africans. J Clin Hypertens (Greenwich) 15(6):413–419. doi:10.1111/jch.12104
81. Bjorkqvist J, de Maat S, Lewandrowski U, Di Gennaro A, Oschatz C, Schonig K, Nothen MM, Drouet C, Braley H, Nolte MW, Sickmann A, Panousis C, Maas C, Renne T (2015) Defective glycosylation of coagulation factor XII underlies hereditary angioedema type III. J Clin Invest 125(8):3132–3146. doi:10.1172/JCI77139
82. Speletas M, Szilagyi A, Csuka D, Koutsostathis N, Psarros F, Moldovan D, Magerl M, Kompoti M, Varga L, Maurer M, Farkas H, Germenis AE (2015) F12-46C/T polymorphism as modifier of the clinical phenotype of hereditary angioedema. Allergy. doi:10.1111/all.12714
83. Pare G, Kubo M, Byrd JB, McCarty CA, Woodard-Grice A, Teo KK, Anand SS, Zuvich RL, Bradford Y, Ross S, Nakamura Y, Ritchie M, Brown NJ (2013) Genetic variants associated with angiotensin-converting enzyme inhibitor-associated angioedema. Pharmacogenet Genomics 23(9):470–478. doi:10.1097/FPC.0b013e328363c137
84. Demirturk M, Polat N, Guz G, Gurdal A, Altun I, Gelincik A, Toz B, Oflaz H, Colakoglu B, Dal M, Buyukozturk S (2012) There is an increased risk of atherosclerosis in hereditary angioedema. Int Immunopharmacol 12(1):212–216. doi:10.1016/j.intimp.2011.11.013
85. Lopez-Lera A, Cabo FS, Garrido S, Dopazo A, Lopez-Trascasa M (2013) Disease-modifying factors in hereditary angioedema: an RNA expression-based screening. Orphanet J Rare Dis 8:77. doi:10.1186/1750-1172-8-77
86. Bouillet L, Mannic T, Arboleas M, Subileau M, Massot C, Drouet C, Huber P, Vilgrain I (2011) Hereditary angioedema: key role for kallikrein and bradykinin in vascular endothelial-cadherin cleavage and edema formation. J Allergy Clin Immunol 128(1):232–234. doi:10.1016/j.jaci.2011.02.017
87. Cai S, Dole VS, Bergmeier W, Scafidi J, Feng H, Wagner DD, Davis AE 3rd (2005) A direct role for C1 inhibitor in regulation of leukocyte adhesion. J Immunol 174(10):6462–6466
88. Cimbollek SH, Gonzalez-Quevedo T, Diaz Fernandez M (2005) CA 125: marker of serosal inflammation in hereditary angioedema? Allergy 60(6):835–836. doi:10.1111/j.1398-9995.2005.00792.x
89. Joseph K, Tholanikunnel BG, Kaplan AP (2002) Activation of the bradykinin-forming cascade on endothelial cells: a role for heat shock protein 90. Int Immunopharmacol 2(13–14):1851–1859