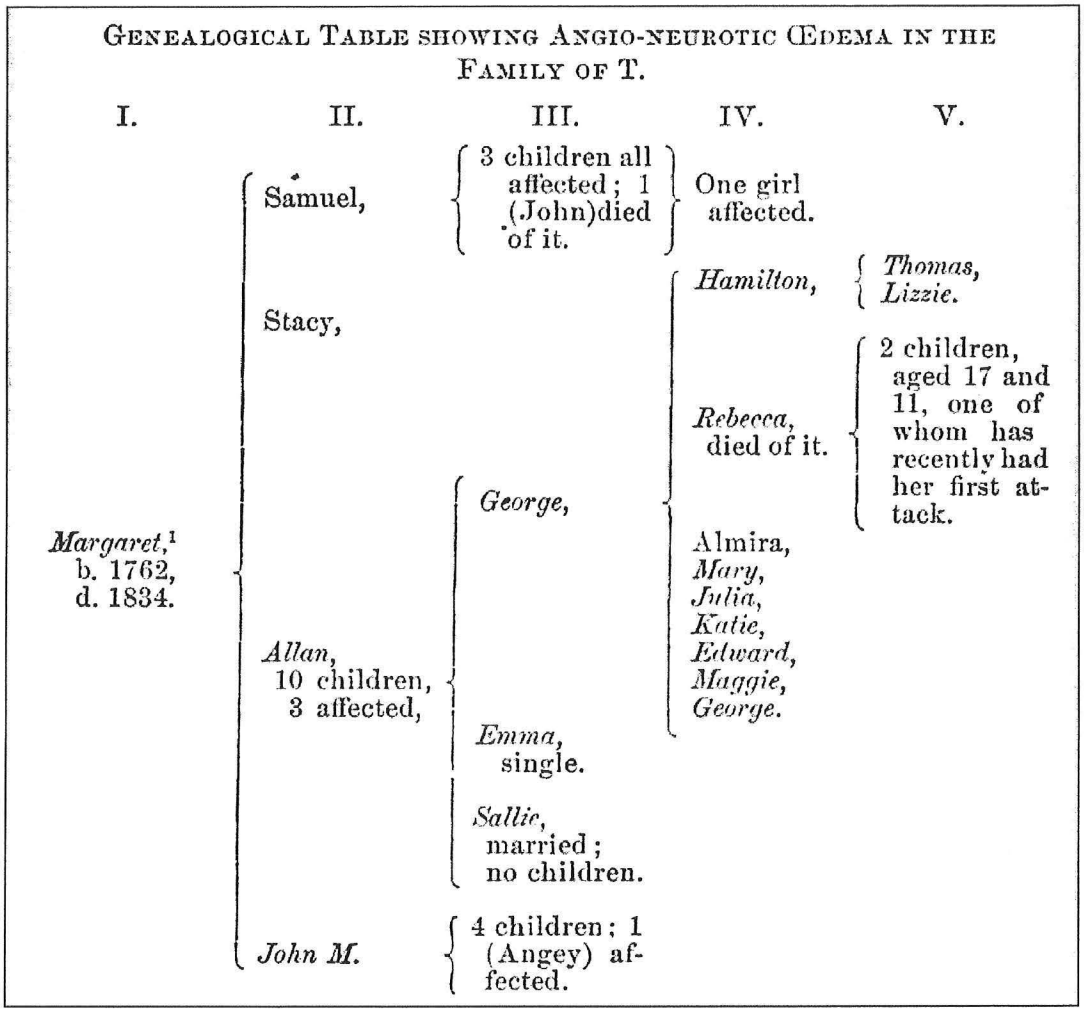
Hereditary Angioedema:

What Have We Learned Since Osler?



GENEALOGICAL TABLE SHOWING ANGIO-NEUROTIC ŒDEMA IN THE FAMILY OF T.

I.	II.	III.	IV.	V.
Margaret,[1] b. 1762, d. 1834.	Samuel,	3 children all affected; 1 (John) died of it.	One girl affected.	
	Stacy,		*Hamilton*,	*Thomas*, *Lizzie*.
			Rebecca, died of it.	2 children, aged 17 and 11, one of whom has recently had her first attack.
	Allan, 10 children, 3 affected,	*George*,	Almira, *Mary*, *Julia*, *Katie*, *Edward*, *Maggie*, *George*.	
		Emma, single.		
		Sallie, married; no children.		
	John M.	4 children; 1 (Angey) affected.		

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BIOGRAPHICAL INFORMATION

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HISTORY

Surprisingly, it was not until the late 1800's that descriptions of angioedema first appeared in the medical literature. John Laws Milton has been credited with the first description of angioedema in 1876 which was described as "giant urticaria". [1] He described one of his patients very descriptively: "SO soon as ever she came into the room I recognized the affection, for there lay, across the face from temple to temple, an oblong tumor almost closing both eyes." Six year later, the famous Swiss physician Heinrich Quincke documented acute, circumscribed edema of the skin and introduced the term "angio-neurotic edema". [2]

In 1888, Sir William Osler reported an inherited form of angioedema which he named "hereditary angio-neurotic edema". [3] Of interest, he first reported his findings to the Philadelphia Neurological Society. The initial index case was a 24 yo woman, "Mrs. H". On September 20, 1887, she was admitted to the Infirmary for Nervous Diseases. She noted a history from childhood of "attacks of transient swelling in various parts" including her extremities and face. The more severe attacks were accompanied by "colic", nausea and vomiting. She noted that several members of her family were similarly affected. Osler, was able to obtain "a tolerably clear history of the affection" from the patient's grandfather, "Mr. T" who was described as "a venerable old patriarch of ninety-two with unimpaired vigor of mind and body". In his report, Osler went on to describe 5 generations of affected family members, two whom died of the disease. One patient died of laryngeal edema (oedema glottidis) and was found dead by the time the family physician arrived. Osler made three key observations. First, "The occurrence of local swellings in various parts of the body, face, hands, arms, legs, genitals, buttocks, and throat. Second, "Associated with the oedema, there is almost invariably gastrointestinal disturbance: colic, nausea, vomiting, and sometimes diarrhea." Third, "A strongly marked hereditary disposition…". Osler further commented on the lack of effective therapy (other than "morphia" for abdominal pain) as well as comparisons to a pediatric disease of painfully swollen joints, a purpuric or urticarial eruption, and intense colic which was described by Henoch 14 years earlier.

By the mid-twentieth century, most of the clinical features of hereditary angioedema (HAE) were well known. Attacks of abdominal pain were recognized as an important feature of the disease and these manifestations were attributed to mucosal edema of the gastrointestinal tract, a point that Osler had already surmised. Trauma was a well recognized precipitant of attacks and in many patients emotional stress was recognized as a common trigger.

In 1961, Lepow and colleagues had discovered a naturally occurring inhibitor of C1 esterase, a heat-labile α-globulin, C1 esterase inhibitor (C1-INH). [4] They went on to show that purified human C'1 esterase enhances vascular permeability in guinea pig skin and this phenomenon could be prevented by purified C1-INH. [5] Landerman and colleagues reported a patient with HAE who had decreased amounts of serum inhibitor of kallikrein and suggested that kallikrein might initiate edema formation. [6] Seventy-five years after Osler's description of HAE, Donaldson and Evans described three families with HAE who had no detectable levels of C1-INH, whereas sera from unaffected relatives contained normal amounts. [7](Figure 1) During attacks of superficial swelling, laryngeal edema, or abdominal pain, C1 esterase activity was readily detectable in both serum and plasma.

In the last 50 years, further advances have been made in the understanding of the genetics and pathogenesis of HAE which has led to increasingly effective therapies. In addition to hereditary C1-INH deficiency, acquired forms of C1-INH deficiency (AAE) have been discovered and more recently reported, another form of hereditary angioedema which is not due to C1-INH deficiency but somehow related to estrogen.

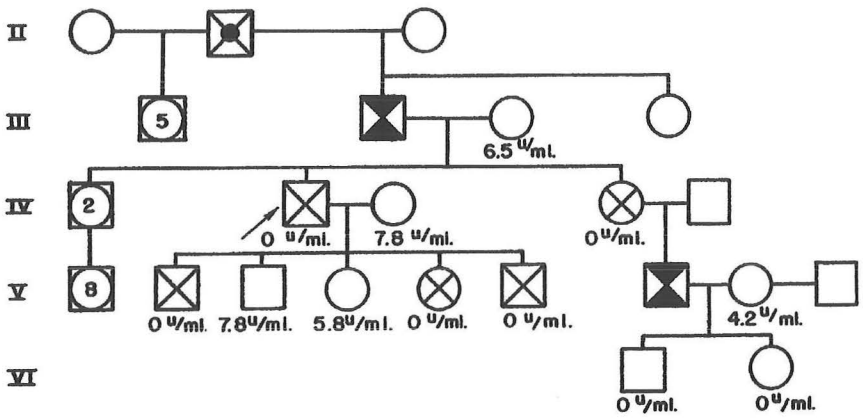


FIG. 1. LEVELS OF SERUM INHIBITOR OF C'1 ESTERASE IN A FAMILY (S) WITH HEREDITARY ANGIONEUROTIC EDEMA. The units of inhibitor of C'1-esterase per milliliter of serum from members of this kindred are enumerated beneath the characters representing those who could be tested. Generations are numbered according to the previous description of this pedigree, which was published by Heiner and Blitzer (9). Square characters designate males; circles represent females. Crossed characters denote those known to have attacks of hereditary angioneurotic edema; those with half of the crossed area in black were persons who are believed to have died during an attack of edema. A black circle within a character indicates death due to causes other than hereditary angioneurotic edema, so far as is known.

Figure 1[7]

CLASSIFICATION OF C1-INH DEFICIENCY AND OTHER HEREDITARY ANGIOEDEMAS

The classification system for HAE and AAE is based on the type of C1-INH deficiency. For HAE, this classification is quite straightforward but for AAE the current classification scheme is somewhat controversial. Recently, hereditary estrogen-sensitive angioedemas have been described and the most recent proposed classification will be discussed.

HAE Type I & II

HAE is a well-defined autosomal trait and is due to deficiency of C1 inhibitor gene production. The disease results from a large number of mutations to the *C1INH* gene. Two types of HAE have been described depending on whether the C1INH gene produces no antigenic protein or a dysfunctional protein. In both types, functional activity of C1-INH is low. Clinically, patients with both types of HAE are identical, however laboratory values of C1-INH levels vary between the two types.

Type I HAE, which accounts for ~ 85% patients, is associated with defective expression of 1 allele and results in low antigenic and functional activity of C1-INH. In type II HAE, ~ 15% patients, the gene product produces a dysfunctional protein but has normal or increased antigenic levels of C1-INH.

Acquired Angioedema (AAE) Types I & II

Angioedema due to an acquired deficiency of C1-INH is a rare form of angioedema first described by Frank Austen and colleagues in 1972 in a patient with lymphosarcoma. [8]. AAE is characterized by increased consumption of C1-INH and hyperactivation of the classical pathway of complement. [9] Clinically, patients with AAE have identical symptoms as those with HAE. AAE

differs from HAE in that there is no family history and that the onset is typically later, usually after the fifth decade of life or later. [10]

Disease	No.	(%)
Lymphatic malignancies	45	(35)
Other malignancies	8	(6)
MGUS	41	(32)
Autoimmune diseases	10	(8)
Other diseases	5	(4)
None	19	(15)
Total	105	(100)

Abbreviations: MGUS = monoclonal gammopathy of uncertain significance.

*Including 105 patients from the literature and 23 from the present report.

Figure 2

AAE is frequently reported in patients with B lymphoproliferative diseases. These lymphoproliferative disorders range from monoclonoalgammopathy of undetermined significance (MGUS) to true malignancies. Neoplastic lymphatic tissue has been shown to consume C1-INH[11] and/or classical pathway complement components. [12] Following these reports of AAE associated with lymphoproliferative diseases, other cases of patients with AAE were reported who had nonhematologic malignancies, autoimmune diseases, and in some cases no disease at all. The diseases and frequency with which they occur are listed in Figure 2 based on a review of the literature of 128 patients. [10]

Autoantibodies that inactivated C1-INH were reported in 1986 in patients with AAE who were otherwise healthy. [13] Based on these observations, 2 separate forms of AAE have been proposed. Type I AAE, paraneoplastic, is mainly associated with lymphatic malignancies. Type II AAE, autoimmune, is caused by autoantibodies to C1-INH.

This classification scheme has been challenged. [14, 15]. Cleaved C1-INH has not been found in all patients with autoimmune AAE. [16] In addition, autoantibodies to C1-INH are commonly found in HAE patients with MGUS[16] Cicardi et al have also shown that half of their patients with AAE due to malignancy had detectable autoantibodies to C1-INH at some time in the course of their disease.[10] They also demonstrated that of 18 patients with autoantibodies, only 4 were healthy: 1 had echinococcus infection, 10 had MGUS and 3 had malignancies. Collectively, these data suggest that AAE with autoantibodies is not a separate disease from AAE in the setting of malignancy. The artificial separation of AAE into two categories is probably inappropriate since in many patients autoantibodies and B-cell lymphatic disease coexist.

The actual risk of malignancy with AAE is not entirely clear but certainly the risk of B-cell malignancy is elevated in patients with AAE. Cicardi reviewed the literature on AAE in 2003 and found 45% patients reported had malignancy. This was much higher than their own series of AAE seen at their institution in Milan, Italy in which 22% patients had malignancy. [10] It is possible that reporting bias might account for the higher frequency of malignancy-associated AAE reported in the literature. Furthermore, based on their series of 23 patients, MGUS was the most frequent associated disease occurring in 57% (13/23) of patients. With a mean follow-up of 8 years, none had progressed to myeloma suggesting that the risk of malignancy is not higher in AAE as compared to other MGUS patients.

Estrogen-dependent and estrogen-associated inherited angioedema

In 2000, Binkley and Davis reported a family who had histories of episodic HAE-like angioedema. [17] The family originated from Southern Italy but members and their descendants reside in Canada and Italy. Angioedema attacks consisted of extremity, face or laryngeal edema and episodic severe abdominal pain and nausea in several members. Several unique aspects were identified in this family. First, the disease only occurred in women, and specifically episodes only occurred during pregnancy, with oral contraceptive use, or with estrogen therapy. Symptoms began

typically within 14-21 days after conception or 5-14 days after endogenous estrogen therapy and lasted 48-72 hours. One patient poignantly recalled "My period was just a day or two late, but the one side of my face swelled up and I knew I must be pregnant, because it was just like what happened to my mother and sisters when they were pregnant." In affected individuals, symptoms occurred with all pregnancies and with each course of estrogen therapy. There were 8 affected women from 3 generations with 1 obligate male carrier. The transmission was consistent with an autosomal dominant inheritance.

Laboratory evaluation for HAE were all normal including complement levels, C1-INH antigenic and functional levels, prekallikrein, , factor XII and HMW kininogen during asymptomatic states. One patient became pregnant and symptomatic and was studied while symptomatic and found to have normal C1-INH antigen and function. [18] Investigations into the *C1INH* gene revealed no abnormalities in the coding sequence or in the 5' regulatory region. Another pedigree with similar features has also been reported. [19] To date, the mechanisms responsible for increased estrogen levels causing angioedema is unknown. This syndrome is currently referred to as *estrogen-dependent inherited angioedema.*

Bork and colleagues also reported in 2000 on 36 women from 10 independent families who had recurrent angioedema with normal C1-INH protein and function. [20] They referred to this type of HAE as type 3 HAE. Eighty-nine percent of these women had episodes of both angioedema and abdominal symptoms and none had urticaria. In contrast to the group of patients reported by Brinkley and Davis, only 1/36 of these women had attacks exclusively during pregnancy. In 10/36, attacks occurred more commonly with oral contraceptives but were not limited to just these times. The other women apparently had angioedema unrelated to estrogen or pregnancy. The term *estrogen-associated inherited angioedema* has been suggested for these women who had attacks not exclusively related to increased estrogen. [21]

PATHOPHYSIOLOGY

C1-INH Structure and Function

Protease	Proportion of plasma inhibitory capacity provided by C1 inhibitor
Complement system	
C1r	100%
C1s	100%
MASP1	*
MASP2	*
Contact system	
Plasma kallikrein	42–84%
Coagulation factor XIa	47%
Coagulation factor XIIa	90%
Fibrinolytic system	
Tissue plasminogen activator	§
Plasmin	§

Figure 3 [23]

C1-INH belongs to the family of serine protease inhibitors (serpin family) which includes α1-antitrypsin and antithrombin III. Unlike most other serpins which have only 1 or a few target proteases, C1-INH is the major inhibitor for several proteases including: 1) C1s and C1r (classical complement pathway), 2) mannin-binding lectin-associated serine proteases (MASPs), 3) FXIa,

FXIIa, and kallikrein (contact system proteases). [22] In addition, C1-INH interacts with other proteases including thrombin, plasmin, and tissue-type plasminogen activator (tPA).(Figure 3)

Mature C1-INH consists of 4789 amino acids and is composed of an N-terminal domain (113 aa) and a serpin domain (365 aa). The structure of the serpin domain is similar to other serpins and is key to the inhibitory capacity of C1-INH. C1-INH inhibits proteases by binding to their active site via its reactive center. Initially, a reversible complex is formed between C1-INH and the target protease. C1-INH then undergoes a conformational change caused by insertion of its reactive center into a 5-stranded β-sheet which results in a modified C1-INH. This modified C1-INH binds tightly to the protease forming a very stable complex. Most of these complexes are removed from the circulation by receptors specific for complexed serpins ensuring efficient removal of active proteases.

The hepatocyte is the primary source of C1-INH, although a number of other cell types, including peripheral blood monocytes, microglial cells, fibroblasts, endothelial cells, the placenta, and megakaryocytes also synthesize and secrete the protein both in vivo and in vitro. [24] INF-γ, INF-α, IL-6 and TNF–α can stimulate the synthesis of C1-INH in a variety of cell types. Based on this, it is not suprising that C1-INH is also an acute phase reactant and may increase 2-fold during uncomplicated infections. [22]

Mechanisms of Angioedema in HAE

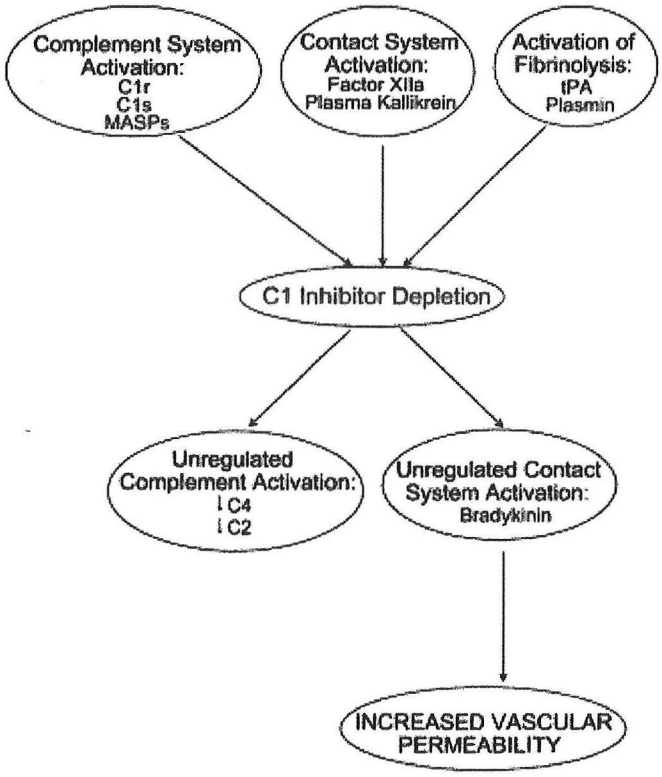


Figure 4 [23]

As discussed earlier, C1-INH regulates the classical and MBL pathways of complement activation, the contact system, and the intrinsic pathway of coagulation. Patients with HAE have evidence for activation of complement and the contact systems primarily. Typically patients with HAE have low C4 and C2, but normal C3 values even while asymptomatic. The low C4 and low C2 results from uncontrolled activation of activated C1. Activation of C3 is typically low or absent, presumably due to efficient control of activation at the level of C4 by C4 binding protein and Factor I.[22] Activation of the contact system typically occurs during attacks as demonstrated by findings of decreased levels of prekallikrein (a substrate for FXII) and reductions in HMW kininogen (a substrate for plasma kallikrein). [25] Increased amounts of cleaved HMW kininogen are found in the vast majority of patients with HAE during attacks. [26, 27] C1-INH has only a limited effect on coagulation. Clinical observations do not support that C1-INH deficiency leads to increased risk of thromboembolic disease, although some thrombin may be generated in attacks. [27] C1-INH is a relatively weak inhibitor *in vitro* of plasmin and tPA, [22] nonetheless one can find evidence of elevated plasmin formation (as determined by plasmin-α2 antiplasmin complexes) during attacks in HAE patients. [26]

Clinically, acute attacks of HAE are triggered by trauma and emotional stress. Factor XII can be activated by phospholipid microparticles *in vivo*. [28] Damaged or apoptotic cells may generate such microparticles, and one could postulate that this could occur with minor trauma, explaining why trauma is a trigger for HAE. Whether microtrauma occurs with emotional stress is unclear and the pathophysiologic basis for stress as a trigger is unknown.

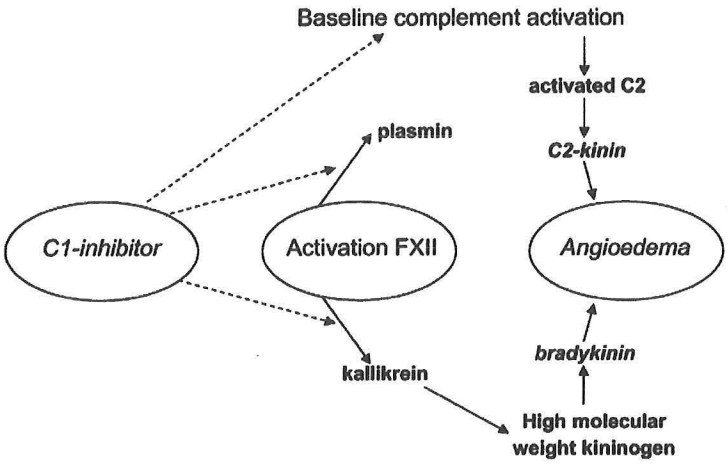


Figure 5 [22]

For decades, the mediators and mechanisms responsible for HAE has been the subject of debate. The two mediators which have been debated the most include bradykinin and a peptide referred to as C2-kinin.(Figure 5) While not completely resolved, the majority of evidence points towards the generation of bradykinin as an important feature in C1-INH deficiency. The contact system of coagulation is activated *in vitro* on contact with negatively charged surfaces. Activation of the contact system yields FXII and kallikrein, both of which are inhibited by C1-INH. Kallikrein can cleave HMW kininogen to form cleaved HMW kininogen and bradykinin. Bradykinin is a nonapeptide with potent vasodilatory activity and enhances vascular permeability.

The measurement of bradykinin in plasma is difficult due to its rapid degradation by carboxypeptidases and requires samples to be collected with special inhibitor cocktails to prevent degradation. Nussberger and colleagues performed key experiments to elucidate the role of bradykinin in HAE. In 1998, they reported a study on 22 patients with HAE, 4 with AAE and 1 with ACE-I related angioedema and compared them to 22 healthy controls. [29] The geometric mean plasma bradykinin concentration in the healthy volunteers was 2.2 fmol/mL (SD 2.2), compared with 3.9 fmol/mL (3.7) among patients with HAE during remission (p=0.095) and 10.4 fmol/mL in the patients with AAE. During acute attacks of edema, in both HAE and AAE, plasma bradykinin rose to 2-12 times the upper limit of normal. Infusion of C1-esterase inhibitor, immediately lowered bradykinin concentrations. In the patient receiving the ACE-inhibitor, bradykinin concentration was very high at 47 fmol/mL during an acute attack of angioedema, but normal at 3.2 fmol/mL after withdrawal of the drug.

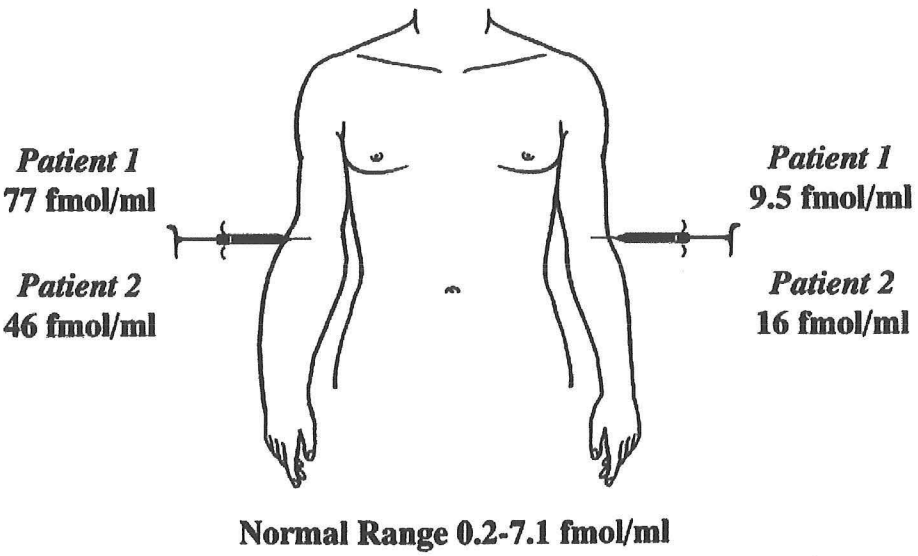


Figure 6

To determine whether the bradykinin measured in plasma was due to systemic activation of the contact system or due to local activation with subsequent systemic leakage they performed an interesting series of experiments in two patients with HAE. [30] In 2 patients with HAE who presented with forearm angioedema, bradykinin levels were measured from the

edematous area and from the contralateral unaffected forearm. As seen in Figure 6, while bradykinin levels were increased in the systemic circulation (contralateral arm), levels were much greater, 3-8 fold higher, in the blood draining the edematous arm. These data suggest that bradykinin generation is local depending on activation of the kinin-releasing system.

Despite this evidence, several arguments exist to oppose bradykinin as the sole explanation for angioedema. [22] First, bradykinin is a very strong vasodilator and one of the most potent hypotensive agents known, yet hypotension is not a feature of HAE. It is possible that since most bradykinin is generated locally, systemic effects may not be seen. Nussberger and colleagues suggest that the lungs may efficiently inactivate bradykinin to minimize or prevent any systemic effects. [30] Second, local injection of bradykin causes pain whereas most patients with HAE have fairly painless angioedema (with the exception of gastrointestinal mucosal edema which is typically very painful). Third, activation of the contact system does not explain why antifibrinolytic agents are efficacious in the treatment of HAE.

C2-kinin has been debated as the other putative mediator in HAE. This peptide is thought to be generated by cleavage from C2b by plasmin. Activation of complement, the contact system (FXIIa), and the fibrinolytic system (plasmin) was required to generate C2-kinin. [31] Since C2-kinin is not a vasodilator it would explain why HAE patients do not have hypotension and angioedema is not painful. Unfortunately, the biologic properties of this peptide are not well established, receptors for it have not been identified, and there are no data on concentrations of C2-kinin in patients with HAE. Recently Kaplan has put the data on C2-kinin into perspective. [32] "However, 27 years have passed and no peptide has been isolated, no assay developed, and neither they nor anyone else has ever reproduced the observation." "Nevertheless, the totality of the data we have and the absence of any progress in characterizing a kinin derived from C-2 should lead to the conclusion that the original observation is more likely incorrect than simply questionable."

In summary, the preponderance of data suggests that bradykinin is an important mediator in HAE and it is unclear if C2-kinin even exists. The efficacy of bradykinin receptor antagonists may shed further light on the role of bradykinin. It is possible that other, unknown vasoactive mediators may also have a role in the pathogenesis of HAE.

ACE-I Angioedema as a human model for HAE

The incidence of angioedema to ACE-inhibitors (ACE-I) is estimated to occur in 1-7/1000 patients and this risk is higher in African-Americans compared to Caucasians. [22] ACE-I angioedema is often unrecognized as its manifestation may occur anywhere between a few hours to 10 years after an ACE inhibitor is first taken. [33] The role of bradykinin in ACE-I angioedema is fairly well established. Nussberger and Cugno reported that bradykinin levels were elevated in HAE patients during attacks and patients with ACE-I angioedema but not in patients with urticaria and angioedema. [34] Gainer et al. performed a double-blind study evaluating the effect of icatibant, a bradykinin-2 receptor antagonist on the short-term effects of ACE inhibition on blood pressure and plasma renin activity. [35] In this study, icatibant administration significantly attenuated the hypotensive effects of captopril and eliminated the increase in plasma renin activity that occurred after the administration of captopril alone. These data confirm that bradykinin contributes to the short-term effects of ACE inhibition on blood pressure in normotensive and hypertensive persons and suggest that bradykinin also contributes to the short-term effects of ACE inhibition on the renin–angiotensin system. In addition, results from a study with a new drug, omapatrilat, that inhibits both ACE and neutral endopeptidase (NEP) indicated that angioedema occurred more frequently in subjects receiving omapatrilat than enalapril. [36] NEP has been shown to metabolize bradykinin to an inactive form. [37] A combination of ACE and NEP inhibition would be expected to reduce bradykinin degradation and could therefore lead to angioedema in sensitive

individuals. Interestingly, rates of angioedema were higher in African-Americans (5.5%) receiving the dual inhibitor vs. only 1.6% in those treated with only the ACE-I. [38] To shed light on racial differences in risks of angioedema to ACE-I, Gainer et al. performed a study comparing the wheal response to intradermal injection of bradykinin in normotensive and hypertensive African-Americans and Caucasians. [39] African Americans had, on average, 70% larger wheal sizes than did the Caucasian group and hypertensive subjects had greater wheal sizes than normotensives.

In ACE-inhibitor associated angioedema, contrary to what happens in HAE , the high plasma bradykinin concentrations are not accompanied by the presence in plasma of cleavage products of HMW-kininogen (the precursor of bradykinin). The pathogenetic mechanism of ACE-inhibitor associated angioedema is likely on the catabolic site of bradykinin metabolism. When ACE is inhibited, aminopeptidase-P (APP) plays a major role in bradykinin metabolism. Adam et al reported lower plasma concentrations of APP in subjects with a history of ACE-I angioedema. [40] These data suggest that low plasma concentrations of APP could be a predisposing factor for development of angioedema in patients treated with ACE-I.

In patients with ACE-I angioedema, angiotensin II receptor blockers (ARBs) are often used as alternative medications. While initially this was perceived as a safe alternative since ARBs do not increase bradykinin levels, literature surveys have suggested approximately 1/3 of patients with ACE-I angioedema will develop angioedema to ARBs. [41] Recently, a mechanism to explain this paradox has been reported. In 2004, Tan et al. reported that increased concentrations of angiotensin II, such that would occur with treatment with an ARB, caused a 3-fold increase in bradykinin receptors. [42] Therefore, increased availability for bradykinin binding might lead to angioedema in susceptible individuals.

Mouse Models of C1-INH Deficiency

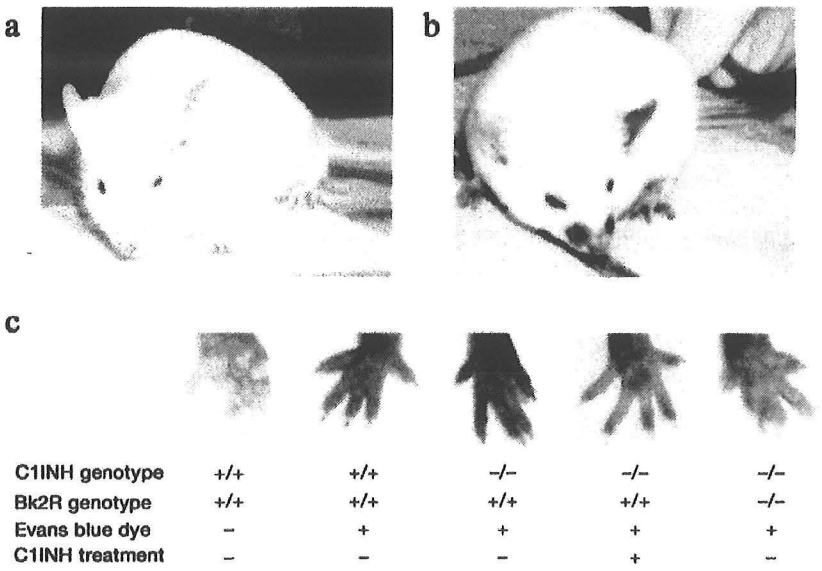


Figure 7 a) WT mouse b) C1-INH -/- mouse c) paws showing extravasation of dye

In 2002, Han et al. published their work on a C1-INH knockout mouse. [43] Heterozygosity for C1-INH deficiency in humans results in HAE, and there has been only one report of a human homozygous for C1-INH deficiency.[44] Han et al. developed homozygous- and heterozygous *C1nh* (gene for murine C1-INH)-deficient mice. C1-INH-deficient mice showed no obvious phenotypic abnormality. Interestingly, these mice, with few exceptions, have not been observed to have typical angioedema attacks. However, following injection with Evans blue dye, both homozygous and heterozygous C1-INH-deficient mice revealed increased vascular permeability in comparison with wild-type littermates.(Figure 7) This increased vascular permeability was reversed by treatment with intravenous human C1-INH, with the plasma kallikrein inhibitor DX88, and with the bradykinin type 2 receptor (Bk2R) antagonist icatibant. In addition, treatment of the C1INH-deficient mice with the ACE-I captopril, increased the vascular permeability. Further experiments were performed in which *C1nh* knockouts were crossed with bradykinin receptor-2 (Bk2R) deficient mice. The offspring mice with

deficiency of both C1INH and Bk2R, demonstrated diminished vascular permeability in comparison with C1INH-deficient and Bk2R-sufficient mice. These murine data support the hypothesis that angioedema is mediated by bradykinin via Bk2R.

Genetics of C1-INH Deficiency

The *C1NH* gene maps to chromosome 11, specifically 11q12-q13.1. [45] It consists of 8 exons distributed over a DNA stretch of 17 kb, with introns containing 17 repetitive Alu sequences148. The structural abnormalities in the *C1NH* gene in patients with HAE are very heterogeneous. *C1NH* gene defects include: large deletions or, less frequently, partial duplications involving Alu repeats distributed along the *C1NH* gene; deletions resulting from a peculiar consensus sequence or an alternative secondary structure; and mutations based on Cytosine-phosphate-guanine (CpG) methylation and subsequent cytosine deamination to thymine. [22] At least 150 mutations have been reported in unrelated patients, with pathogenic amino acid substitutions distributed over the entire length of the coding sequence. [46-48] All of these mutations lead to an apparent failure to synthesize or secrete functional C1-INH protein. In almost all cases of HAE-I and HAE-II, defective expression has been related to impaired protein secretion, transinhibition of C1-INH translation, or extensive consumption. [22] However, on the basis of most clinical data, disease expression cannot be attributed to specific mutant proteins. Variable clinical presentation is thought to result from genetic or nongenetic elements distinct from the *C1NH* gene.

Cases of angioedema with no family history but with functionally low levels of C1 inhibitor and recurrent attacks are often observed. Pappalardo et al. studied their population of HAE patients from Italy. [49] Among 137 independent kindreds followed for HAE, 45 (32.8%) patients with early onset of the disease were registered as sporadic cases. Nineteen patients with unaffected parents were screened for point mutations and microdeletions-insertions. Among the 19 patients studied at the DNA level, 9 de novo single nucleotide substitutions and 6 de novo microdeletions were found. De novo exon deletions were detected in 3 additional patients with Southern blot analysis. Based on their data, de novo C1inhibitor mutations and exon deletions account for at least 25% of all unrelated cases of angioedema. This finding has implications relevant to the genetic epidemiology and genetic counseling of HAE.

Numerous databases contain information on *C1INH* gene mutations. Due to the rapid changes in information, a mutation database was created (http://hae.biomembrane.hu) with the following purposes: 1) to help the collection of information on genetic alterations of the C1 inhibitor (*C1INH*) gene discovered in several laboratories with different motivations; 2) to create a database where these data can be searched and compared according to several characteristics; 3) to provide additional comprehensive help in the difficult decision that a new mutation may be disease causing or not; and 4) to give informative help for research efforts closely or more distantly related to *C1INH* genetics. [48] To date, a total of 150 mutations have been recorded including 22 gross mutations, and 128 micro-mutations.

CLINICAL FEATURES

Signs and Symptoms

HAE has two common clinical manifestations, angioedema and abdominal symptoms. These symptoms are episodic, and typically involve a single site but may have contiguous spread. Angioedema and abdominal symptoms may occur independently or in association with one another. In a series of 72 HAE patients reported by Frank et al., 96% had angioedema of the extremities, 85% had

involvement of the face, and 64% the oropharynx. In the same series, 93% had recurrent abdominal pain with 88% reporting accompanying nausea and vomiting and 22% with watery diarrhea.

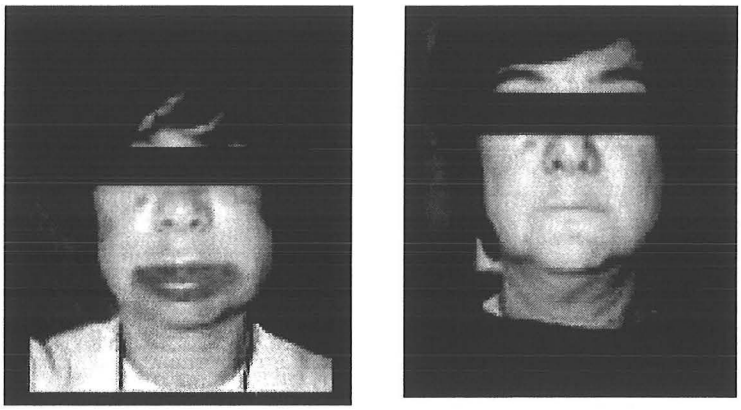


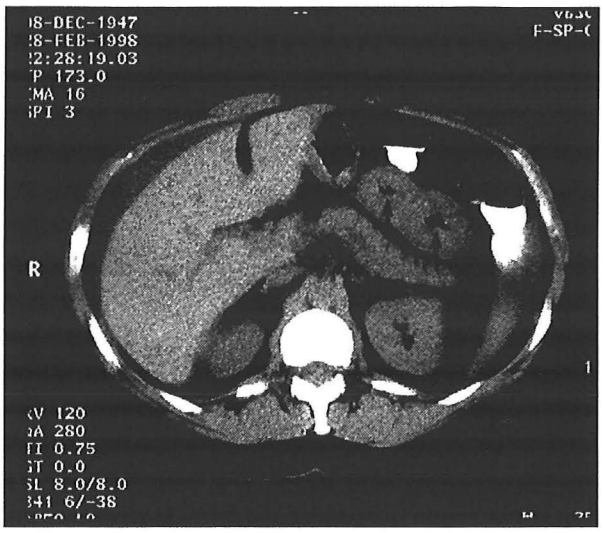
Figure 8 [50]

Angioedema most commonly involves the face, extremities, genitals, and larynx. The angioedema of C1-INH deficiency is identical in appearance to other forms of angioedema. The skin edema is nonpitting, not erythematous, non-pruritic and has ill-defined margins. It is typically painless, but many patients do complain of a "tightness" due to stretching of their skin. Angioedema episodes typically last 2-3 days but may be as brief as 4 hrs or persist up to 7 days. [51]

As opposed to most other forms of angioedema in which accompanying urticaria are typical, HAE is not associated with urticaria. This is a key diagnostic feature in that the presence of urticaria virtually excludes a diagnosis of C1-inhibitor deficiency. Nonetheless, there are other cutaneous rashes that may accompany HAE which may be mistaken for urticaria. In a survey of US patients, Frank et al., noted that 26% of 72 HAE patients may have a variety of erythematous rashes including erythematous mottling, erythema marginatum, or erthema multiforme. [51] These rashes are mild and transient and usually preceded or accompanied the angioedema attacks but in some cases occurred independent of the attacks.

Laryngeal edema is a life-threatening complication of HAE. Half of patients have at least one lifetime episode of laryngeal edema. Bork et al. recently described clinical features of laryngeal edema in a cohort of 123 HAE patients. [52] Sixty-one (49.7%) patients experienced a total of 596 laryngeal edema episodes. The ratio of laryngeal edema episodes to skin swellings and abdominal pain attacks was approximately 1:70:54 in patients who had laryngeal edema. The mean age at the first laryngeal edema was 26.2 years. The mean interval between onset and maximum development of laryngeal edema was 8.3 hours. Laryngeal edema may occur at any age, although young adults appear to be at greatest risk. In contrast to allergic angioedema which peaks within minutes, in adults with HAE, the interval between onset of symptoms and acute risk of asphyxiation is usually long enough to allow for use of appropriate emergency procedures. In the past, up to 30% of patients with HAE died due to laryngeal edema. [51] With improvement in emergency services, and in some countries, the availability of effective therapies, fatalities are much less common today. Nonetheless, fatalities still do occur. Bork et al. reported on 6 deaths due to HAE in Germany in the 1990's. [53] Three patients were undiagnosed at the time of their fatal asphyxiation. The interval between onset of the laryngeal edema and asphyxiation was 20 minutes in a 9-year-old boy, and in the other patients, the interval was 1 to 14 hours (mean for all, 7 hours). The possibility that the first episode of laryngeal edema may be fatal must be emphasized to the relatives of HAE patients.

Recurrent abdominal pain due to bowel wall edema is another common clinical manifestation of HAE occurring in 70-80% of patients. [22] This is another distinguishing feature in that other forms of angioedema, rarely are associated with abdominal pain. Gastrointestinal symptoms of HAE include abdominal pain which may range from mild to very severe, intractable pain and can be associated with nausea, vomiting, and diarrhea. One of my patients with HAE has also gone through labor pains as well as episodes of nephrolithiasis. She rates the pain of her HAE attacks to be much worse than either labor pains or the pain from her nephrolithiasis! In some patients, these gastrointestinal symptoms along with



plasma extravasation and vasodilatation can lead to hypovolemic shock as reported by Cohen et al. [54] They proposed that patients with HAE who present with abdominal pain, hypotension, hemoconcentration, and leukocytosis form a distinct subgroup with a high risk of hypovolemic shock. Ascites has also been reported in patients with HAE and is thought to be due to a combination of effects including fluid extravasation into the peritoneum and changes in the splenoportal axis. [55, 56] Both ultrasound and CT imaging have confirmed bowel wall edema.(**Figure 9**) [57]

Due to the clinical similarities between abdominal angioedema and an acute abdomen, many patients undergo unnecessary exploratory laparotomies. Patients with acute HAE attacks may have physical findings such as moderate tenderness, normal to increased and high pitched bowel sounds and in some cases rebound tenderness. Moderate-marked leukocytosis (up to 31,000/mm^3) may occur with HAE further confusing the picture. [58] While ultrasounds and CT scans may demonstrate edematous intestinal mucosa and the presence of free peritoneal fluid, these signs are not specific for angioedema. A history of current or recent body angioedema would be much more suggestive of an attack of HAE. Therapies that are specifically effective for acute HAE may be helpful in differentiating angioedema from other abdominal emergencies.

In addition to cutaneous, abdominal and laryngeal edema there are rare case reports of HAE patients experiencing edema in other areas. Frank et al. reported 2/72 patients with radiographically demonstrated transient pleural effusions with cough and mild pleuritic chest pain with peripheral angioedema episodes. [51] A case report from the French literature describes a patient with HAE requiring mechanical ventilation due to pulmonary edema but this appears to be an anomaly. Cicardi has proposed that the lung is protected due to inactivation of bradykinin. Studies comparing the physiological effets of intravenous vs. intra-arterial injections of bradykinin have suggested over a 95% pulmonary clearance rate of bradykinin. [59] While rare patients have been reported in older literature to have had seizures or hemiparesis, [6, 51] postulated to be due to cereberal edema, this has not been confirmed. Van Dellen and Myers reported a case of a patient with bladder involvement in HAE. [60] The patient had documented HAE and presented with episodes of gross hematuria . Cytoscopic examination revealed raised hemorrhagic lesions in the vesical walls and biopsies of the lesions showed submucosal edema. The lesions resolved when the angioedema was better controlled.

Triggers of HAE Attacks

Local trauma and emotional stress are the two most common triggers for attacks of patients with HAE. In Frank et al.'s series, trauma precipitated attacks in 54% of patients while 43% had attacks attributed to emotional stress. [51] A very common cause of trauma is dental manipulation which may trigger oropharyngeal or laryngeal edema. Tonsillectomy and other accidental traumas are also common triggers. Other activities that may precipitate extremity edema include: typing, prolonged writing, pushing a lawn mower, hammering, and prolonged standing. Horseback riding and sexual intercourse can precipitate buttock and genitalia swelling. While both trauma and stress are common triggers, HAE patients can clearly have spontaneous angioedema. Allergic triggers are not a feature of HAE. Interestingly, many patients may have a prodrome of tingling or tightness at a site that may precede the actual edema formation by hours.

It is currently known that HAE is influenced by the fluctuation of female hormones, but the effects sometimes appear to vary greatly among women. Estrogen has been shown to increase fibrinolytic proteins, alter the contact system, decrease C1-INH levels and increase bradykinin. [22] There is general agreement that women taking supplemental estrogens, particularly in the form of oral contraceptives may have increased attacks of HAE, [61, 62] and estrogen containing contraceptives are not recommended in HAE patients. Nevertheless, the course of some women's disease seems to be unaffected by estrogen. Progesterone may also have a role in influencing attacks of HAE. The onset of HAE attacks often coincides with the beginning of adolescence, or the incidence of attacks can increase during puberty, perhaps due to the elevation in the progesterone level in the luteinizing phase of the menstrual cycle. Visy et al. performed a study to evaluate the relationship between serum levels of sex hormones and the incidence of HAE attacks. [63] They noted that 13 out of 21 female patients experienced an increase in the frequency of attacks at menarche. Overall, the number of attacks was significantly higher OR= 6.36 in females with high progesterone levels and was even higher OR=13.4 for subcutaneous attacks only. The authors suggest that progesterone levels may useful in predicting the severity of the future clinical course of HAE. In regards to pregnancy, the data are conflicting. Frank et al. in their U.S series noted improvement of HAE during the last trimesters of pregnancy, while Agostini et al. in their larger Italian series did not find any reduction with pregnancy.

Age of Onset

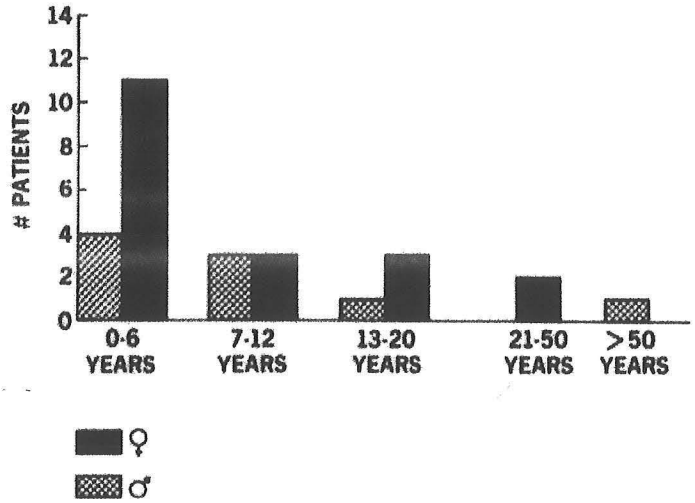


Figure 1. Age of onset of hereditary angioedema in 28 patients. In most cases symptoms began in childhood.

Figure 10 [51]

C1-INH deficiency is present at birth but only rarely manifests in the first few years of life.[64, 65] About half of patients become symptomatic before the age of 10, and another 1/3 by the age of 20. [22] This early onset of angioedema may be a helpful diagnostic aid as most idiopathic angioedema typically manifests in adulthood. While HAE typically manifests in childhood, there are case reports of patients becoming initially symptomatic in their 50's. [51, 66] There does not appear to be any difference between age of onset amongst males and females. Asymptomatic adults with C1-INH deficiency have been described who were detected by family screening of an affected family member and represent 5% of patients in large series. [62]

Frequency of Attacks

Figure 8

The frequency of attacks varies greatly in HAE. This variability is seen between patients and also exists within a patient who may be asymptomatic for years and then have a series of attacks. In a survey of the Italian case series, amongst untreated patients, slightly less than 1/3 of patients have attacks more than once per month, 40% have 6-11 episodes/year and 30% range from rarely

symptomatic to completely asymptomatic. [22] Perhaps surprisingly, the range of clinical severity and frequency has no correlation with C1-INH concentrations and can vary tremendously amongst families.

(Figure 11) [51]

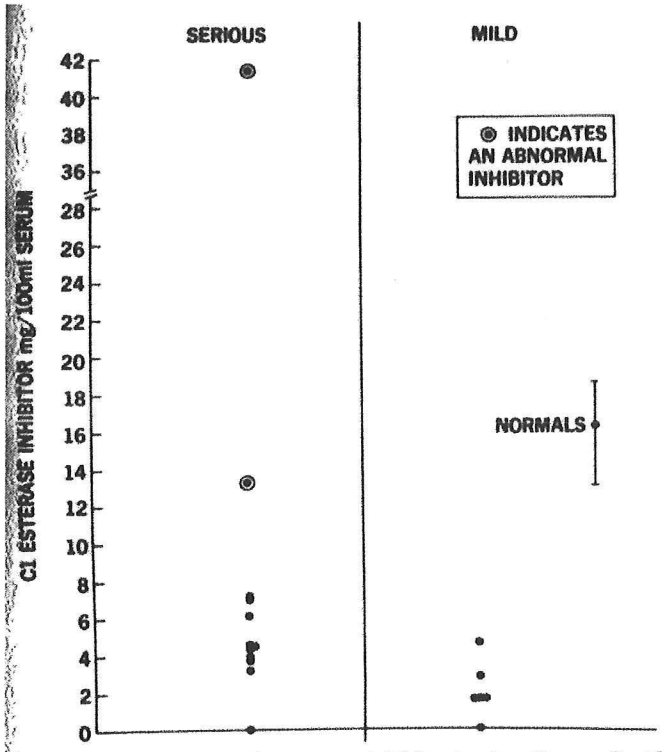


Figure 8. Correlation of C1 esterase inhibitor levels with severity of disease. Patients with the most serious and the mildest disease are shown. There is no correlation between the inhibitor level and the severity of disease.

It has been postulated that other genetic or environmental effects may influence the clinical manifestations of HAE. Lung et al. reported on a polymorphism of the bradykinin 2 receptor involving a 9 bp deletion found in exon 1 of the gene. [67] They found that 17 patients with symptomatic HAE were either homozygous -/- or heterozygous +/- for the deletion. Two patients aged 9 and 18 were homozygous +/+ and asymptomatic but had decreased C1-INH levels and affected family members. The authors hypothesized that the ability of bradykinin to induce angioedema may, in part, be determined by the hB2BKR exon 1 genotype. Nonetheless, due the young age of these patients it is possible that they will become symptomatic later and further conformational studies are needed.

Farkas et al. evaluated the potential role of *H. pylori* in HAE attacks. [68] They assessed the frequency of *H. pylori* infection in 65 patients with HAE serologically and using the carbon-14-urease breath test in patients with positive serology. Nineteen of 65 patients had *H. pylori* infection. All 19 patients with infection, and 11 of 46 without infection, had a history of recurrent episodes of acute abdominal pain and the frequency of abdominal symptoms was significantly higher in the infected group ($p=0.002$). *H. pylori* infection was successfully eradicated in 18/19 patients. In nine of 19 patients with dyspepsia, the frequency of angioedema episodes decreased from 100 over 10 months before eradication to 19 during the 10-month follow-up period. Based on these findings, evaluation and treatment for *H. pylori* may be reasonable in HAE patients with frequent abdominal attacks.

Disease Associations with HAE

Most patients with HAE are otherwise healthy. However there are several reports of autoimmune diseases in patients with HAE. [51, 69-75 76-79] Frank and colleagues systematically evaluated 157 HAE patients for manifestations of autoimmunity. [70] Nineteen of these patients (12%) had clinical immunoregulatory diseases including glomerulonephritis (5), Sjogren's syndrome (3), inflammatory bowel disease (3), thyroiditis (2), systemic lupus erythematosus (1), drug-induced lupus (1), rheumatoid arthritis (1), juvenile rheumatoid arthritis with IgA deficiency (1), incipient pernicious anemia (1), and sicca syndrome (1). All eight patients with HAE who developed an autoimmune disease with a known human histocompatibility antigen association, developed a disease associated with their histocompatibility antigen haplotype ($p = 0.014$). Although only four patients developed Sjogren's syndrome or sicca syndrome, an additional nine manifested part of the sicca complex. They also found patients with HAE with features suggestive of an immune-based abnormality idiopathic pancreatitis (3), Raynaud's disease (2), partial lipodystrophy (1), chronic chorioretinitis (1), and alopecia universalis (1).

DIFFERENTIAL DIAGNOSIS

The diagnosis of HAE is fairly straightforward when patients present with symptoms of episodic angioedema and abdominal pain as well as a family history of angioedema. While a family history may be lacking in about 1/3 of patients, the vast majority of patients have both angioedema episodes and abdominal symptoms. While other forms of angioedema are more common than HAE, they rarely have associated abdominal symptoms. Thus, any patient with episodic angioedema (without urticaria) who has episodes of abdominal pain should be evaluated for C1-INH deficiency. Urticarial vasculitis is another relatively uncommon condition that may be confused with HAE. Patients with urticarial vasculitis may have both angioedema as well as abdominal pain. Furthermore, a subset of these patients also have low levels of complement. Distinguishing features of urticarial vasculitis include the presence of urticarial lesions, though they differ from typical urticaria in that they may be non-pruritic, bruising, and lesions may persist for a few days. In those patients with urticarial vasculitis with hypocomplementemia, in addition to low C4 and C2 levels, they usually have depressed C3 and CH50 levels whereas these are normal in HAE.

Patients with HAE may have just episodic angioedema without abdominal pain. In the absence of a family history, there are still features of HAE that are somewhat different than other forms of angioedema. With the exception of ACE-I induced angioedema which is also bradykinin-mediated, other forms of angioedema typically have histamine as a major mediator. Thus typical idiopathic or allergic angioedema will often respond somewhat to antihistamines, whereas antihistamines have no effect in HAE. The time from onset to peak of angioedema is typically hours in HAE versus minutes in most other forms of angioedema. The majority of patients with histamine mediated angioedema will also have associated urticaria. As stated earlier, the age at onset of angioedema occurs earlier in HAE patients. Given all of the above, it is uncommon to find patients that by history alone, the diagnosis is obscure. According to Frank et al. "...the main stumbling block to the proper diagnosis of hereditary angioedema is its omission as a diagnostic consideration." [51] In fact in their initial series of 30 patients reported in 1976, the average length of time to make the diagnosis after the onset of symptoms was 21 years. Whether our diagnostic acuity has improved in the last quarter century is unknown, but in my experience, there is still a delay of many years before a proper diagnosis is made.

LABORATORY DIAGNOSIS

The labs which are most commonly used to evaluate patients with HAE are C4, C1-INH concentration, and C1-INH function. An additional lab, C1q, is helpful in determining AAE. Each type of HAE or AAE has a somewhat different laboratory profile which will be discussed later.

	C1-INH level	C1-INH function	C1q	C4	C3
HAE-I	↓	↓	**N**	↓	**N**
HAE-II	**N or ↑**	↓	**N**	↓	**N**
EDIAE	**N**	**N**	**N**	**N**	**N**
AAE-I	↓	↓	↓	↓	**N or ↓**
AAE-II	**↓ or N**	↓	**↓ or N**	↓	**N or ↓**

Figure 12: EDIAE (estrogen dependent inherited AE)

C1-INH concentration

C1-INH concentration, also referred to as antigenic levels, may be assayed by several different methods including radial immunodiffusion, nephelometry, or ELISA. Based on large sample sizes, the lower limit of normal is 15 mg/dl. [80] Most patients with HAE I have values well below the normal limits, typically < 50% of normal. [81] Since patients with HAE are heterozygotes, one would expect their C1-INH level to be ~50% of normal. In contrast, patients with HAE I have serum levels on average 17% of normal. One mechanism is that due to activation of the classical complement cascade due to lower C1-INH levels, there is increased catabolism of C1-INH by the other systems C1-INH acts as an inhibitor. [81] Recently, Cicardi and colleagues using RT-PCR demonstrated that the normal message (C1-INH mRNA) is reduced by >50% when only one allele is expressed functionally. [82] They also unexpectedly found a similar reduction in HAE II suggesting that the level at which the C1-INH producing cell is expressing a functional protein is somehow used to regulate C1-INH.

Patients with HAE II have normal or even elevated concentrations of C1-INH. Higher levels have been attributed to mutated C1-INH proteins binding to other serum proteins. [22] Treatment of HAE with androgens typically results in only modest if any increase in C1-INH levels.

Patients with AAE may have either normal or low amounts of C1-INH. Normal antigenic amounts may be seen if C1-INH autoantibodies result in cleaved C1-INH which is a nonfunctional but antigenic 96-kd protein. [83]

A word of caution in regards to C1-INH levels. I have seen several patients referred for abnormal C1-INH levels, in which the history was less than compelling. One recurring problem is the fact that not all laboratories use the appropriate reference range. I have seen several patients who do not

have histories consistent with C1-INH deficiency, who had levels of C1-INH of ~ 20 mg/dl and was reported by LabCorp as below their normal range. Testing for C1-INH function was always normal and in some cases, C1-INH levels from other labs was also normal. Finally, I have seen several instances where the lab reports from "outside" vendors were transposed with erroneous reference ranges or units when inputted into our clinical data repository.

C1-INH Function

C1-INH function may be assessed by several different methods including both enzyme-based chromogenic assays and ELISAs. Enzyme-based chromogenic assays may use different substrates including Et-CO-Lys or MeCO-Lys. Different types of ELISA tests are also available and measure either C1-INH-C1r or C1-INH-C1s complexes. Depending on the assay, normal ranges for C1-INH function are typically > 68-80%. [22] C1-INH function is an extremely sensitive assay and virtually all patients with all types of HAE and AAE have decreased C1-INH function. Curiously, data from a Hungarian angioedema center reported that 30% of 72 sera samples from 36 untreated HAE patients had normal C1-INH functional levels. [22] This data is clearly at odds with all other centers. False positive tests for C1-INH function have been reported to be fairly high with ranges of 54-90%. [80] The authors of this report suggested this is due to degradation of the sensitive enzyme used in the assay during prolonged transit of samples to the lab. Due to the higher false positive rates, chromogenic assays have a low positive predictive value of only 36%. Assays using C1-INH-C1s ELISA have reportedly a PPV of 92% (Vargas et al. unpublished data). [22] These ELISA assays may be normal when patients with HAE are receiving treatment with C1-INH concentrate. [22]

C4 Concentration

C4 levels may be determined by nephelometry or radial immunodiffusion. Functional C4 may be assessed using a hemolytic assay but is not used in clinical practice. As with C1-INH function, all patients with both HAE and AAE usually have low levels. C4 levels have been advocated as a screen by many and some case series have indicated all patients to have low C4 levels. [84] Nevertheless, not all patients with HAE have low C4 levels while asymptomatic, but during an attack, a normal C4 virtually excludes a diagnosis of HAE. [51] While C4 levels do not correlate with severity, serial C4 levels may be of some use in an individual patient as they may indicate disease activity. Similar to C4, C2 levels are also decreased in patients with HAE but these tests are not as readily available and are not used routinely clinically.

As mentioned previously, diminished C3 levels are extremely rare in HAE, and when present is only minimally depressed. Likewise, CH50 is only occasionally decreased in HAE. In contrast, patients with AAE often have undetectable CH50. [85]

C1q Concentration

In HAE, C1 or its subcomponents are typically normal. In contrast, patients with AAE typically have significantly decreased C1q levels. Patients with AAE have accelerated catabolism of C1-INH with resultant hyperactivation of the classical complement cascade. Studies of patients with AAE have shown increased catabolism of both C1-INH and C1q. [9] Confusing the picture is the fact that some patients with AAE may have normal values of C1q. Fremeaux-Bacchi et al. reported 19 patients with AAE, all of whom had anti-C1-INH antibodies, in which 4/19 had normal levels of C1q. [85] Patients with low C1q also had undetectable CH50 levels whereas the patients with normal C1q had normal levels of CH50. Patients with hypocomplementemic urticarial vasculitis may also have decreased C1q and C4, and occasionally decreased C1-INH due to consumptive loss.

Diagnostic Strategies

Various algorithms for the diagnosis of HAE and AAE have been recently proposed. [22, 50] None of the algorithms take into account a careful history which is the most helpful diagnostic strategy. As discussed earlier, there are several clinical features that are very suggestive of C1-INH deficiency including episodic angioedema with characteristic features (slow to peak, triggered by trauma), recurrent abdominal pain, and in 2/3 of HAE cases, a family history. When patients present with these characteristic features, obtaining C4 and C1-INH function levels would be appropriate. If both tests are normal, C1-INH deficiency can be excluded. If the C4 is low, but C1-INH function is normal, hypocomplementemic urticarial vasculitis should be considered. If both C4 and C1-INH function are low, C1-INH deficiency is confirmed and further tests can be performed. If there is a family history, a C1-INH quantitative level could be obtained to determine whether the patient has HAE I (low C1-INH level) or HAE II (normal C1-INH level). Practically, the value in determining this is unlikely to be of much clinical use at the present time. In the absence of family history and if the patient is over the age of 40, determining a C1q level and obtaining an SPEP would be appropriate. A depressed C1q level in the setting of low C1-INH function would be diagnostic of AAE. Even if the C1q is normal, as discussed earlier, the patient may still have AAE and if the SPEP does not reveal paraproteinemia, further evaluation for occult malignancy should be entertained. Unfortunately, anti-C1-INH antibodies are not commercially available, though arguably the presence or absence of anti-C1-INH antibodies is of questionable clinical relevance.

The laboratory evaluation of HAE has recently been investigated in a systematic fashion to provide sensitivity and specificity data. [80] The combination of a low C4 and low C1-INH function has a 98% specificity and 96% negative predictive value. The authors caution that the accuracy of diagnosis is significantly improved when repeat samples confirm the results.

It should be stressed that these laboratories often require repeating, particularly when the results are only marginally abnormal. Another good general rule would be not to order C1-INH testing of any sort in patients with histories of urticaria. C1-INH quantitative levels should not be ordered initially, unless one orders them with C1-INH functional tests as patients with both HAE II and AAE II may have normal C1-INH quantitative levels. Furthermore, since these tests are ordered rarely, there is typically a great deal of confusion amongst laboratory personnel in actually ordering the correct test (quantitative vs. functional C1-INH). It is imperative to ensure one obtains the proper test results before making the diagnosis.

CURRENT TREATMENT IN THE U.S.

Treatment of HAE is separated into 3 management issues. First, is treatment of acute attacks, which currently is very minimal in the United States due to unavailability of effective therapies. Second, long-term preventative therapy aimed at reducing the frequency of attacks. Lastly, since patients with HAE have trauma as a potential trigger, short-term prophylaxis prior to certain procedures is a separate management issue.

Management of Acute Attacks

In the US, supportive therapy is the mainstay for acute attacks. [86] The affected area of angioedema will determine what types of therapy are required. Upper-airway edema that may progress to laryngeal edema can be life-threatening. Monitoring of the airway is the critical factor. Patients with severe airway edema typically have preceding symptoms of dysphagia and change in voice tone. In patients who have pharyngeal, tongue or symptoms of laryngeal edema (dysphonia, throat tightness, drooling etc) it is recommended that someone skilled in the management of upper airway emergencies

(otorhinolaryngologist or anesthesiologist) be readily available to assess and intervene if necessary with intubation or tracheostomy. Personnel who are not skilled at intubating, may worsen local angioedema via their traumatic attempts which can further activate the contact system cascade. Once intubated, patients typically remain intubated for 24-72 hours. Fortunately, airway edema typically evolves over hours so if the patient presents in a timely fashion, arrangement for appropriate personnel can be made. Most airway edema peaks over several hours and begins to recede after 36 hours. [51] Epinephrine either locally or systemically may be used, but is generally ineffective, but may help reduce edema transiently. Corticosteroids which are a mainstay of treatment for other forms of angioedema are likewise generally ineffective.

Patients who have abdominal attacks typically require analgesia, anti-emetics and fluid resuscitation. Abdominal pain may be severe and usually requires parenterally administered narcotics. Some patients with HAE become addicted to narcotics and may present to the emergency room frequently with feigned abdominal attacks requesting narcotics. These patients are uncommon and very difficult to manage. Unfortunately, there is no supportive therapy for extremity swelling and these are usually not treated.

Several other therapies may also be considered for acute attacks of HAE but most of these are relatively ineffective or potentially risky. The use of fresh frozen plasma (FFP) is controversial. FFP appears to be effective in acute therapy since it serves as a source for C1-INH. In 1969, Pickering et al. reported on their treatment of 2 patients, 1 with laryngeal edema, who had apparent rapid improvement in their symptoms as well as an increase in inhibitor function. [87] The authors concluded that we "should encourage the use of fresh-plasma therapy for potentially life-threatening or painful episodes in H.A.E. until an effective purified form of the C'1-esterase-inhibitor becomes available." Unfortunately, as will be discussed later, over 36 years later, C1-INH replacement therapy is still not available in the U.S. While many advocate its use, Frank and Rosen in the US, recommend avoiding use of FFP acutely based on personal observations as well as those of others of worsening of attacks when FFP is given acutely. [88] They note that FFP may serve as a substrate for further kinin-generated edema formation. Finally, FFP carries the risk of transmitting blood-borne diseases.

Antifibrinolytics such as epsiolon-aminocaproic acid (Amicar) have also been advocated as a treatment option. Cicardi and colleagues have anecdotally noted "satisfactory results" with high doses of tranexamic acid (1 g every 3-4 h) if given early into an attack. No controlled studies have been performed for any anti-fibrinolytic for acute HAE. Since tranexamic acid is not available in the U.S., Amicar may be administered as an IV bolus of 5 gm over an hour followed by 1 g/hr for the next 10 hrs. This therapy generally does not result in major benefits. [51] Androgens are quite effective for prophylaxis of attacks, but are rarely helpful for acute attacks, likely due to their prolonged onset of action.

Long-term Prophylaxis

Long-term preventive therapy is typically advocated for patients with relatively frequent episodes (> 1/month) or less frequent but more severe episodes. Attenuated androgens and anti-fibrinolytics are the mainstays of preventive therapy in the U.S. with androgens being typically favored due to their greater efficacy.

Attenuated Androgens

Spaulding is credited with the first use of androgens in patients with HAE. [89] He empirically treated 6 members of one family with methyltestosterone in a double-blind fashion and noted improvement and suggested that the drug thickened collagen fibers thus preventing swelling. Independently, Frank and colleagues noted that patients with hereditary angioedema who were taking

high-estrogen birth control pills often become more severely ill and that sometimes therapy could be limited to simply stopping birth control pills. [88] Their first attempt at hormonal treatment, although never published, was a double-blind study of high-dose progesterone therapy reasoning that HAE is often much less severe in the later stages of pregnancy and after delivery often becomes more severe. Progesterone increases in the later stages of pregnancy and decreases at the time of delivery. They found no statistically significant effect on attack frequency, although the patients all reported that they felt better on progesterone therapy. They became interested in testing an agent that would inhibit estrogen synthesis in men and women, and decided upon danazol, an agent developed as a contraceptive. In 1976, they published their study of danazol 200 mg tid vs placebo in which patients took a course of study medication for 28 days or until they had an attack. [90] Nine patients completed 93 courses (47 placebo and 46 danazol). They observed dramatic results with danazol with attack rates of only 1/46 courses (2.2%) on danazol vs. 44/47 (94%) for placebo! They also found that C1-INH levels rose threefold (4/9 reaching nl or near nl levels) and C4 levels increased 15-fold though most remained low. Since then, androgens have been widely used and long-term experience has been gained. It has become appreciated that the doses required for prevention are typically much less than was originally used.

TABLE 8. Long-term prophylaxis in patients with hereditary angioedema

Drug	No. Patients	Mean Length (yrs ± SD)	Effectiveness* (% Patients)
Tranexamic acid	27	1.8 ± 0.8	28
Androgen derivatives	59	4.8 ± 3.4	97

* Reduction in frequency of attacks >80%.

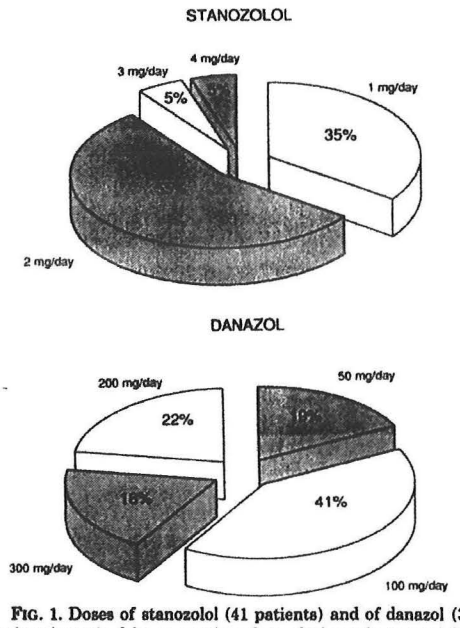


FIG. 1. Doses of stanozolol (41 patients) and of danazol (30 patients) required for prevention of attacks in patients receiving long-term treatment for hereditary angioedema.

Figure 13 [62]

Danazol and stanozolol are the two most commonly used androgens for HAE. Methyltestosterone is an alternative as well as oxandrolone (Oxandrin) which has also been reported to be efficacious in case reports. [91] Danazol (Danocrine) is typically effective in doses of 50-200 mg daily or every other day. While some protocols suggest initial high dose therapy with tapering afterwards[92], others favor starting with a lower dose and titrating upwards. [93] I typically prefer the latter approach. Stanozolol (Winstrol) is used in much lower doses and is typically effective at 0.5-2 mg daily or alternate days. Stanozolol is currently not available in the U.S. since the manufacturer Sanofi Synthelab ran into manufacturing problems and decided to discontinue manufacturing the drug. The U.S. Hereditary Angioedema Association has made arrangements for patients to purchase the drug from pharmacies equipped to make the drug in-house. Comparative dosages are lacking between danazol and stanozolol.

Contraindications to androgen use are pregnancy, lactation, prostate cancer, and childhood, though several reports of apparent safe use of androgens in children exist. [65, 91, 93, 94] A number of potential side effects exist with androgen therapy including hair growth, weight gain, acne, voice deepening, vasomotor symptoms, decreased breast size, menstrual irregularities, decreased libido, hepatic necrosis or cholestasis, altered liver enzymes, liver neoplasms (hepatocellular adenomas[95, 96] or carcinomas), hypertension, atherogenesis with altered lipid metabolism, polycythemia, and hemorrhagic cystitis. [50] Recently Széplaki et al. compared lipid profiles between danazol-treated patients with HAE and 2 control groups (patients who did not receive long-term danazol prophylaxis and untreated healthy subjects). [97] Serum concentrations of HDL and apolipoprotein A-I were significantly lower, whereas

LDL and apolipoprotein B-100 were higher in the danazol-treated patients compared with the 2 control groups.

Despite this lengthy list of adverse effects, androgens are reasonably well-tolerated in HAE patients. Several reports of long-term therapy with androgens have been published, many with over 10 years of follow-up. [98, 99 100-102] The most recent long-term report from the Milan group, reports doses that most patients currently receive and provides data for both danazol and stnozolol. [102] They followed 70 patients for a median of 10 years and compared adverse effects to 33 patients who never received long-term androgen therapy. The most frequent side effect was menstrual irregularities seen in 50% of premenstrual women on danazol vs. 18% with stanozolol. Increase in body weight occurred in 28% with danazol vs. 17% with stanozolol. Both effects were dose related. Disturbingly 25% of androgen treated patients compared to 3% controls had hypertension. The authors note that stanozolol is probably more effective than danazol since it has less adverse effects and facilitates patients achieving a therapeutic dose. No controlled studies comparing the efficacy of danazol and stanozolol exist and it is unlikely they will ever be performed.

Recently an International Concensus has made recommendations for the monitoring of HAE patients treated with androgens. [50] Every 6 months the following labs are recommended: CBC, liver function tests, lipid profile, and urinalysis. In adults receiving ≤200 mg androgen (presumably danazol), annual liver spleen ultrasonography is suggested. For adults on doses 300-600 mg/d or prepubertal patients, ultrasonography is recommended every 6 months.

Antifibrinolytic Agents

In the U.S. ε-aminocaproic acid (EACA or Amicar®) is the only available antifibrinolytic for use while in other areas, tranexamic acid is available. In 1972, Frank et al. performed a double-blind study of EACA 15 g/d vs. placebo in 5 patients with > monthly attacks of HAE. [103] Four of 5 patients had marked responses with no attacks while on EACA but several attacks on courses of placebo. Subequently, they determined that while very effective in high doses, there were significant adverse effects such as myalgias (25%), myositis, postural hyopotension and fatigue. [51] Doses of 8-10g/d (in divided doses) are typically therapeutic and much more tolerable, while doses less than this are generally ineffective. Since this is an antifibrinolytic agent, there is a concern for thrombosis, though in the treatment of HAE this appears to be uncommon. Tranexamic acid has less side effects than EACA and is the preferred agent in countries where both are available. [50] Based on clinical experience of large patient series, antifibrinolytics are felt to be less effective than androgens with 70% responding somewhat but only 30% of patients having a considerable reduction in the number and severity of attacks. [22] In general these agents are typically reserved for patients failing androgens (usually due to androgenic side effects) but are more commonly used in pediatric patients. In my experience, these agents while well tolerated are cumbersome (20 pills/day), expensive and generally ineffective.

Short-Term Prophylaxis

Since trauma is a well known inciting event for patients with HAE, procedures such as dental work, endoscopy, intubation and surgery are thought to increase the risk of angioedema. Short-term prophylaxis procedures prior to these events have been used but reports are limited to case reports or small case series. Some patients with HAE do not have angioedema after undergoing surgery without pretreatment and others may have angioedema with some dental procedures but not all. Given this inherent variability, it is difficult to assess the true efficacy of these prophylactic regimens without larger numbers or controlled studies.

Farkas treated 12 patients with HAE with danazol 600 mg/d for 4 days before and 4 days after dental procedures (predominantly extractions) and measured C4, C1-INH and CH50 levels after the

procedures. [104] None of the patients had any angioedema but all showed evidence of lower complement and C1-INH levels. Others have used stanozolol at doses of 4 mg every 6 hrs for 5 days prior to dental or surgical procedures. [99] It should be noted that transient abnormalities in liver enzymes are common at these high doses. Current international concensus recommendations are for danazol at 600 mg/d for 5 days before and 2 days after procedures. [50]

Fresh-frozen plasma has also been used as prophylactic therapy for various surgical procedures. Atkinson reported on their experience using FFP prophylaxis in 53 patients with HAE undergoing all types of dental treatment over a ten-year period. Only 3 of 45 patients (6.7%) covered with FFP had a minor angioedema attack after dental therapy in 10 yr. No attacks of moderate or severe swelling were seen. Wall et al. noted similar results with only 2/30 cases receiving FFP prophylaxis developing mild complications. [105] FFP is typically given as 2 units the night before surgery. Due to concerns with blood-borne infections solvent/detergent-treated FFP has been recommended when available. [50] EACA has also been used in some patients successfully starting therapy 2-3 days prior to surgery. [51] Tranexamic acid has also been used as prophylaxis in some patients. [106]

Treatment of AAE

The course of acquired C1-INH deficiency can be related to the course of the underlying disease. Treatment of the underlying disease has been shown in several cases to reverse the biochemical and/or clinical abnormalities of acquired C1-INH deficiency. [85, 107, 108] Analogous to HAE, patients with acquired C1-INH deficiency have received attenuated androgens for prophylaxis and as will be discussed later, C1-INH concentrate to treat acute attacks. Nevertheless, these patients are frequently resistant to attenuated androgens, whereas they tend to benefit from antifibrinolytic agents. [62, 109] According to Cicardi, antifibrinolytics are more effective for long-term prophylaxis in this population and represent the first choice for patients with acquired C1-INH deficiency. [10]

As will be discussed later, replacement therapy with C1-INH plasma concentrate is the treatment of choice (where available) for life-threatening laryngeal attacks. However, patients with AAE partially resistant to this treatment have been reported. [22] The response to treatment may differ from HAE because of the rapid catabolism of C1-INH that characterizes AAE. Higher doses of C1-INH plasma concentrate were required in patients with AAE than HAE. [110] Other therapies that bypass the problem of accelerated C1-INH catabolism, such as kallikrein inhibitors and bradykinin-2 receptor antagonists (see below) may be other therapeutic alternatives in AAE patients.

FUTURE THERAPIES FOR HAE

C1-INH Replacement Therapy

C1-INH preparations are purified and pasteurized or vapor-heated concentrates from pooled human plasma and have a half-life of 64 hours. [22] C1-INH concentrate has been effective in all 3 phases of HAE therapy including acute attacks, and short and long-term prophylaxis. In Europe, C1-INH concentrate has been widely used since 1973. [111] Early studies used C1 inhibitor that was partly purified from pooled plasma. Intravenous administration of the concentrate during acute abdominal or laryngeal attacks of hereditary angioedema in five patients resulted in abatement of symptoms in addition to increased serum C4 activity. [112] Unfortunately, the early use of C1-INH concentrate was associated with a significant risk of transmission of the human immunodeficiency virus (HIV) and hepatitis. From 1983 to 1984, 13 patients developed a non-A, non-B hepatitis related to the administration of C1-INH concentrate, probably because of a contaminated batch. [62]

Since the introduction of viral inactivation steps, transmissions of infectious agents have not been reported. [113] A lyophilized C1 inhibitor concentrate, which is vapor-heated to 60°C for 10 hours

under pressure, conditions that effectively inactivate HIV and hepatitis B and C viruses, was subsequently developed. A double-blind placebo controlled study evaluating onset of action of vapor-heated C1-INH concentrate, revealed in 11 patients with 55 attacks that 71% of abdominal attacks, 75% laryngeal, 100% face, and 56% extremity attacks responded in 30 minutes. [114] In the placebo arm, no abdomen, larynx or face attacks responded in 30 minutes and only 6% of extremity attacks responded in 30 minutes. Overall, the time from the start of an infusion to the beginning of improvement in symptoms was shorter for the C1 inhibitor infusions than the placebo infusions (55 vs. 563 minutes, $P<0.001$). Another larger randomized controlled study of 22 patients confirmed that relief was almost twice as fast in persons receiving C1 inhibitor concentrate than in the controls: 7.62 hours versus 15.35 hours. [115] A large open-label observational study evaluated 42 HAE patients with 517 episodes of laryngeal edema. [116] Eighteen patients received 500- or 1000-U injections of C1-INH concentrate in 193 episodes. C1-INH concentrate was effective in all laryngeal edemas. The interval from injection to interruption in progress of symptoms ranged from 10 minutes to 4 hours.(Figure 14) The mean ± SD duration of laryngeal edema was 15.3 ± 9.3 hours in patients who received C1-INH concentrate and 100.8 ± 26.2 hours in those who did not.(Figure 15)

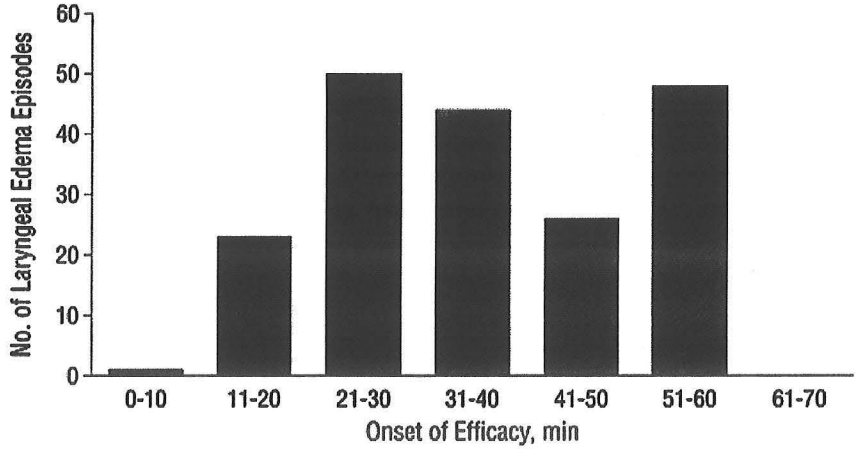


Figure 14

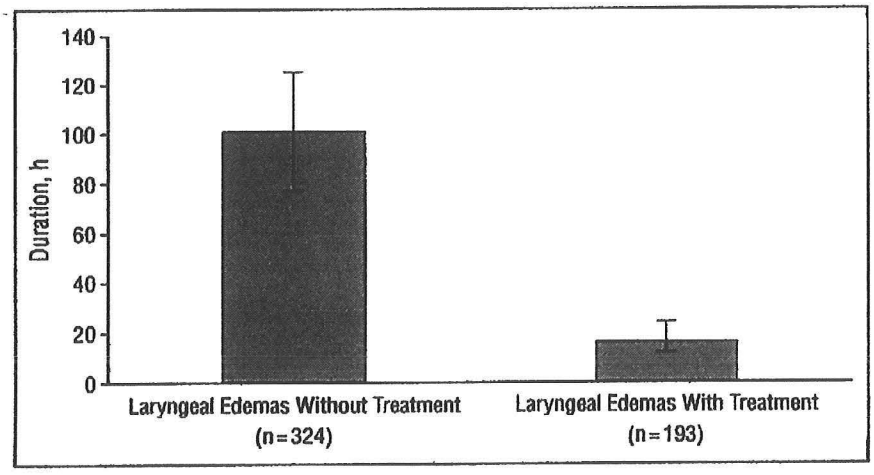


Figure 15

C1-INH concentrate has also been used for prophylaxis. Data on both short and long-term prophylaxis is limited. Data published in abstract form is available from Martinez-Saguer et al. on 30 HAE patients with frequent attacks (every 1-7 days) who were treated with 500–1000 U of C1-INH concentrate (Berinert® P) as a bolus injection intravenously twice or three times a week. [117] The frequency of attacks was markedly reduced in all subjects under prophylaxis and > 75% of the subjects were almost free of symptoms. Side effects of C1-INH concentrate are rare and include fever and headache. To date, the formation of autoantibodies to C1-INH as a result of C1-INH concentrate administration has not been observed in any patient with HAE. Information on C1-INH concentrate used in short-term prophylaxis is limited to a few case reports. [118, 119]

Plasma-derived products are of limited availability for reasons related to their human blood origin, supply, safety concerns, and/or development costs. Due to these concerns, recombinant technology has been applied to the development of C1-INH concentrate. Major advantages of production of human C1-INH via recombinant DNA technology are that the recombinant material is not derived from a human source, that the production can more easily be controlled, and that the production can be scaled up. Expression of wild-type human C1-INH has been successfully achieved in transgenic rabbits, and this recombinant inhibitor is now in phase II clinical development for the treatment of acute attacks in patients with HAE. [22] Expression of C1INH via the mammary gland of transgenic animals was performed in rabbits because of rabbits' short generation time. In addition, a lactating rabbit may produce 10 L milk per year; because of this, transgenic rabbits may yield sufficient quantities of recombinant human C1-INH (rhC1-INH). *Pichia*-expressed C1-INH may constitute another alternative to plasma-derived C1-INH but is still at a very early stage of development. The yeast *Pichia pastoris* is increasingly used as an expression system to produce recombinant proteins on a large scale.

Kallikrein Inhibitors

Kallikrein is known to catalyze the conversion of kininogen to bradykinin and therefore, inhibitors of kallikrein should theoretically be beneficial in treatment of HAE. The kallikrein inhibitor DX-88 was generated by Dyax via phage display, a technique for rapidly identifying target-specific protein binders. The phage display process involves generating many possible binders specific for each desired target, in this case human plasma kallikrein, and then selecting the binder with the highest affinity. [22] DX-88 was selected on the basis of its extremely high binding affinity for human plasma kallikrein. [120] An open-label study of DX-88 in C1-INH deficiency was conducted in 4 centers in Europe. [120] Of the 9 treated patients, 7 patients had HAE, and 2 had AAE. In this dose-ranging study, 3 patients each received 10 mg, 40 mg, or 80 mg intravenous DX-88. One patient had a drug-related anaphylactoid reaction. Patient-reported times to the start of attack resolution ranged from 25 to 240 minutes (mean, 92 minutes); patient-reported times to complete resolution ranged from 2 to 72 hours (mean, 38.6 hours).

On June 30, 2005, more recent data was presented at the World Allergy Congress on data from the EDEMA2 trial (their 3rd Phase II trial). [121] In the analysis, 120 HAE attacks were observed in 47 patients treated with IV DX-88. The median time to clinical response, defined as onset of relief of HAE symptoms within four hours post-dosing, was 30 minutes. Dyax plans to study DX-88 given subcutaneously in ongoing Phase II trials. With regard to safety data, over 365 doses of DX-88 have now been administered to over 165 people and no antibodies to the drug have been detected thus far. DX-88 for use in treating HAE has orphan drug designation in the United States and Europe, and Fast Track designation in the United States.

Bradykinin-2 Receptor Antagonists

Since bradykinin is assumed to be the key mediator of HAE, blocking its effects via the B2 receptor is another strategy for treatment. Icatibant is a specific, competitive peptidomimetic bradykinin B2 receptor antagonist. However, it contains nonproteinogenic amino acids and is not degraded by the 2 main bradykinin cleaving enzymes, carboxypeptidase and ACE. [22] In addition to HAE, it is being evaluated for refractory ascites and severe burns. As discussed earlier, icatibant showed efficacy in reversing increased vascular permeability in the murine model of HAE. Recently, the results of a phase II single-dose open-label trial for the treatment of acute HAE attacks in 15 patients for 20 acute attacks of HAE became available. [122] Eight of 20 attacks were treated with subcutaneous icatibant at either 30 or 45 mg and the others were treated with different doses of IV icatibant. In the patients treated subcutaneously, mean onset of symptom relief was 35 and 27 minutes for the 30 and 45 mg doses respectively. This is substantially shorter than the patient's historical onset of relief which was 30 and 35 hours. Icatibant is currently in Phase II trials in the U.S. It also has orphan drug and Fast Track designation by the FDA.

CONCLUSIONS

Certainly, many advances have been made in the understanding of HAE since the time of Osler. While specific therapies for acute attacks of HAE are not available in the U.S., several promising agents are being developed and have fast track status with the FDA and hopefully will be available in the coming few years. Despite our more thorough understanding of the genetics and pathophysiology of HAE, many questions remain unanswered; particularly those that relate to the inherent variability of disease expression despite similar biochemical or genetic profiles. Our understanding of other forms of hereditary angioedema that are unrelated to C1-INH but are estrogen dependent or associated remains in its infancy. Finally, despite our better understanding of this disease, in clinical practice it remains largely unknown or misunderstood with patients being misdiagnosed or inappropriately treated.

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