Hereditary Angioedema - On Comorbidities and Treatment

Sundler Björkman, Linda

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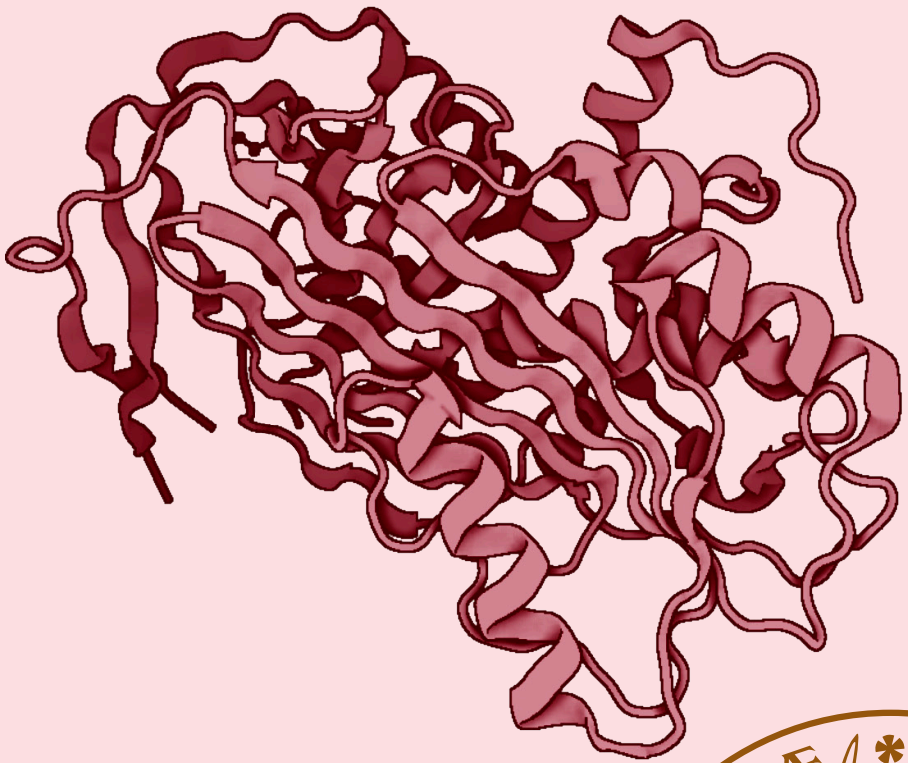
LINDA SUNDLER BJÖRKMAN
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY



Hereditary Angioedema – On Comorbidities and Treatment

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Linda Sundler Björkman

LUND
UNIVERSITY

DOCTORAL DISSERTATION
Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on May 12, 2023, at 13.00 in LUX auditorium, Helgonavägen 3, Lund

Faculty opponent
Professor Johan Rönnelid
Department of Immunology, Genetics and Pathology, Uppsala University

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Abstract:

Hereditary angioedema (HAE) is caused by deficiency or dysfunction of C1 inhibitor (C1INH). The primary manifestation of the disease is recurrent swellings of the skin and mucosal tissues, caused by increased bradykinin production in the contact activation system. C1INH is an important regulatory protein in several physiological pathways, and as a result HAE may be associated with comorbidities. The overall aim of this thesis was to investigate the extent of possible comorbidities in HAE patients, their lymphocyte populations and medical treatment, since these may have bearing on comorbidities. In a registry-based study comparing the Swedish HAE cohort with the background population we found that HAE patients had a higher risk of autoimmune diseases, especially SLE, as well as allergy, asthma and hyperlipidemia. We also found a higher risk of cardiovascular diseases including hypertension, venous thromboembolism (VTE) and arterial thromboembolism (ATE) in patients with HAE. In a second study, further analyses of the data revealed that HAE patients had a higher risk of VTE but not ATE, when evaluating composite VTE and ATE respectively. By using the National Prescribed Drug Register we found that 50% of the HAE patients in Sweden were treated with long-term prophylaxis (LTP); i.e. 30% with C1INH, 10% with androgens and 10% with tranexamic acid. One third of the HAE patients were prescribed the on demand medication icatibant (a bradykinin B2 receptor antagonist). In a fourth study, lymphocyte populations in HAE patients were compared with those of matched healthy controls using flow cytometry. In addition, complement and complement activation fragments were analyzed. The results showed reduced NK cell counts in HAE patients compared with controls and a shift in T cell populations towards a Th2 cell phenotype in HAE patients. These findings also correlated with disease activity in HAE.

Taken together, the studies of this thesis show comorbidities and alterations in lymphocyte populations in HAE. To what extent these depend on the disease itself, the disease activity or may be modified by different treatments used will be important to further elucidate in future studies, especially since novel therapies for long-term prophylaxis were recently introduced, and more are on the way.

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Linda Sundler Björkman

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Faculty of Medicine

Respiratory Medicine, Allergology and Palliative Medicine

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To my family

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List of Papers

This thesis is based on the following papers which are referred to in the text by their Roman numerals I-IV.

I. **Sundler Björkman L**, Persson B, Aronsson D, Skattum L, Nordenfelt P, Egesten A. Comorbidities in hereditary angioedema-A population-based cohort study. Clin Transl Allergy. 2022 Mar;12(3):e12135.

II.

a. Grover SP, **Sundler Björkman L**, Egesten A, Moll S, Mackman N. Hereditary angioedema is associated with an increased risk of venous thromboembolism. J Thromb Haemost. 2022 Nov;20(11):2703-2706.

b. Petersen RS, Fijen LM, Cohn DM. "Hereditary angioedema is associated with an increased risk of venous thromboembolism": comment from Petersen et al. J Thromb Haemost. 2023 Jan;21(1):179.

c. Grover SP, **Sundler Björkman L**, Egesten A, Moll S, Mackman N. "Hereditary angioedema is associated with an increased risk of venous thromboembolism": reply. J Thromb Haemost. 2023 Jan;21(1):180-182.

III. **Sundler Björkman L**, Thulin M, Ekström M, Nordenfelt P, Egesten A. Trends in Treatments With Disease-Specific and Interfering Drugs in Patients With Hereditary Angioedema in Sweden. J Allergy Clin Immunol Pract. 2023 Feb;11(2):621-628.

IV. **Sundler Björkman L**, Elmér E, Egesten A, Skattum L. Th2 predominance and decreased NK cells in patients with hereditary angioedema – a connection with autoimmune disease? (manuscript)

Abbreviations

AAE	acquired angioedema
ACE	angiotensin converting enzyme
AD	autoimmune disease
ANA	antinuclear antibody
APC	antigen presenting cell
APS	antiphospholipid syndrome
APTT	activated partial thromboplastin time
ATE	arterial thromboembolism
BCR	B cell receptor
C1INH	C1 inhibitor
CD	cluster of differentiation
CTLA-4	cytotoxic T lymphocyte associated protein 4
DVT	deep vein thrombosis
ELISA	enzyme-linked immunosorbent assay
HAE	hereditary angioedema
HAEnCI	hereditary angioedema with normal C1inhibitor
HLA	human leukocyte antigen
HK	high molecular weight kininogen
IFN-γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
LTP	long-term prophylaxis
MHC	major histocompatibility complex
NET	neutrophil extracellular trap
NK	natural killer
pdC1INH	plasma derived C1inhibitor
PE	pulmonary embolism
SLE	systemic lupus erythematosus
TCR	T cell receptor
Th cell	T helper cell
Treg	regulatory T cell
TLR	Toll-like receptor
VTE	venous thromboembolism

Abstract

Hereditary angioedema (HAE) is caused by deficiency or dysfunction of C1 inhibitor (C1INH). The primary manifestation of the disease is recurrent swellings of the skin and mucosal tissues, caused by increased bradykinin production in the contact activation system. C1INH is an important regulatory protein in several physiological pathways, and as a result HAE may be associated with comorbidities. The overall aim of this thesis was to investigate the extent of possible comorbidities in HAE patients, their lymphocyte populations and medical treatment, since these may have bearing on comorbidities. In a registry-based study comparing the Swedish HAE cohort with the background population we found that HAE patients had a higher risk of autoimmune diseases, especially SLE, as well as allergy, asthma and hyperlipidemia. We also found a higher risk of cardiovascular diseases including hypertension, venous thromboembolism (VTE) and arterial thromboembolism (ATE) in patients with HAE. In a second study, further analyses of the data revealed that HAE patients had a higher risk of VTE but not ATE, when evaluating composite VTE and ATE respectively. By using the National Prescribed Drug Register we found that 50% of the HAE patients in Sweden were treated with long-term prophylaxis (LTP); i.e. 30% with C1INH, 10% with androgens and 10% with tranexamic acid. One third of the HAE patients were prescribed the on demand medication icatibant (a bradykinin B2 receptor antagonist). In a fourth study, lymphocyte populations in HAE patients were compared with those of matched healthy controls using flow cytometry. In addition, complement and complement activation fragments were analyzed. The results showed reduced NK cell counts in HAE patients compared with controls and a shift in T cell populations towards a Th2 cell phenotype in HAE patients. These findings also correlated with disease activity in HAE.

Taken together, the studies of this thesis show comorbidities and alterations in lymphocyte populations in HAE. To what extent these depend on the disease itself, the disease activity or may be modified by different treatments used will be important to further elucidate in future studies, especially since novel therapies for long-term prophylaxis were recently introduced, and more are on the way.

Background

Hereditary angioedema

Hereditary angioedema (HAE) is a rare genetic disease with a prevalence of 1:50,000, equally affecting men and women in numbers, and with no known differences between ethnic groups. The primary manifestation of the disease is recurrent swellings of the skin and mucosal tissues. The most feared incident is swelling of the larynx, which can be life-threatening in lack of access to necessary treatment. The reason for the swellings is enhanced production of bradykinin, which causes leakage of fluid from blood vessels to the tissues. Bradykinin is produced when the contact system is activated. The disease is caused by low concentration of, or defective function of C1inhibitor (C1INH). C1INH is an inhibitory protein in the complement system, as well as a regulator in the contact activation system, coagulation system and fibrinolytic system. This gives rise to the question if HAE is accompanied by other diseases, evolving from these systems.

History

Already the great Greek physician Hippocrates (377-460 BC) used the term "oedema" to describe swellings of organs. Many centuries later, the term angioedema was described as a medical concept by Quincke in 1882. In 1888, Osler was the first to describe the hereditary aspect of HAE[1]. Several decades later, in 1963, Donaldson discovered that HAE is caused by lack of C1INH[2]. In the mid-60s two variants of HAE were discovered; one caused by deficiency of C1INH, HAE type 1 (HAE1), and one caused by defective function of C1INH, HAE type 2 (HAE2)[3]. In the mid-80s the first cloning of *SERPING1*, the gene coding for C1INH, took place as well as the definition of the protein structure. It was also discovered that the disease is caused by mutations in the *SERPING1* gene[4,5]. Research work in the 1980s and 1990s led to convincing evidence establishing that bradykinin is the mediator generating angioedema[6-9]. Further research led to new knowledge of the interactions between the contact-, complement-, coagulation- and fibrinolytic systems in which C1INH plays an important role[10,11]. In the year 2000, a form of HAE with normal activity and level of C1INH (HAEnCI) was described[12,13]. It is a very rare condition with symptoms similar to those in HAE1 and HAE2. To date, rare variants in six genes have been identified in patients with HAEnCI; Factor XII

(*F12*), angiopoietin-1 (*ANGPT1*), plasminogen (*PLG*), kininogen 1 (*KNG1*), myoferlin (*MYOF*), and heparan sulfate-glucosamine 3- O- sulfotransferase 6 (*HS3ST6*), however the genetic background of the majority of patients with HAEnCI is still unknown[14].

Pathophysiology

HAE1 and HAE2

HAE is an autosomal dominant disease which means a probability that 50% of the offspring of a patient with HAE will inherit the disease. It also means that an individual with only one affected allele will develop the disease. HAE1 is the most common variant affecting around 85% of patients. In HAE1, the mutation leads to decreased concentration of C1INH. In HAE2, affecting approximately 15% of patients, C1INH has a defective function. More than 700 variants in the *SERPING1* gene are described as associated with HAE; missense, nonsense variants, insertions, deletions and splice variants. Up to 25% of these variants represent *de novo* mutations[15-17]. Generally, the level of C1INH in HAE1 is 5-30% of the normal level. C1INH is a member of the serpin (serine protease inhibitor) family[18,19]. The name C1INH derives from its inhibitory effect in the first component of the complement system; C1. C1INH is also a regulatory protein in the contact-, coagulation- and fibrinolytic system. Activation of the contact system leads to excessive formation of bradykinin, that increases vascular permeability and induces swellings[20,21]. Bradykinin is a peptide consisting of nine amino acids and binds to the bradykinin B2 receptor, a G protein-coupled receptor expressed on epithelial cells. When the bradykinin B2 receptor is activated, cadherin molecules between endothelial cells are degraded, leading to vascular permeability resulting in angioedema. In addition to B2 receptors, the first catabolite of bradykinin, des-Arg^{9}-bradykinin, binds to bradykinin B1 receptors. Unlike B2 receptors, B1 receptors are transiently expressed on epithelial cells following tissue injury or other inflammatory stimuli, and receptor binding leads to inflammatory responses[21,22].

HAEnCI

Bradykinin is thought to play a major role in HAEnCI, primarily in patients with mutations in *FI2* and *PLG*. Mutations in *MYOF* and *HS3ST6* are involved in endothelial dysfunction. The exact mechanisms underlying HAEnCI are yet to be characterized[14].

Acquired angioedema

In acquired angioedema (AAE) the level of C1INH is decreased, due to an underlying disease, most often a lymphoproliferative disease, such as monoclonal gammopathy of uncertain significance (MGUS), non-Hodgkin´s lymphoma and

myeloma. AAE may also be associated with autoimmune disease and solid tumours. The pathogenesis is not fully understood but may involve formation of autoantibodies directed towards C1INH which are present in most cases. In approximately 70% of cases, C1q are low. It is a rare condition, with a prevalence of 1:100,000- 500,000[23,24].

Clinical manifestations

The hallmark symptoms of HAE are recurrent cutaneous and submucosal angioedema attacks involving the face, tongue, extremities, genitals, gastrointestinal tract, and larynx. Swellings of the skin and stomach are most common, 90% will be affected by gastrointestinal attacks during their lifetime. These attacks can cause severe pain, nausea, vomiting and diarrhea, sometimes leading to unnecessary surgery[25]. Most often the face is affected, which can give rise to significantly changed appearance which may have a negative impact on quality of life[26,27]. A dreaded complication of HAE is having a swelling in the larynx, affecting 50% of patients during their lifetime. Angioedema episodes generally develop in the course of several hours and last 2-5 days without treatment[28].The attack frequency varies from several attacks per month to several years between attacks. The onset of symptoms in HAE1 and 2 commonly develops in childhood, and usually worsens during puberty[29]. The symptoms vary during lifetime, as well as between family members with the same genetic variant. Prodromal symptoms include a phenomenon called erythema marginatum; a web-like rash that appears at the site of emerging angioedema, affecting around one third of patients[30]. Other prodromal symptoms are a tingling sensation and fatigue, which most patients experience. There are still questions about what initiates an attack, although some triggering factors are known. Among the most common triggers are physical trauma; accidental or associated with dental or medical procedures, psychological stress, infection, physical exercise, alcohol and estrogen, hence exogenous estrogens are to be avoided[31,27]. HAE usually presents with more frequent and severe attacks in women due to exposure to estrogen, affecting kininogenase activities[32].

Diagnosis

Medical history

The diagnosis of HAE1 and 2 is made based on a medical history of recurrent angioedema attacks in the absence of urticaria, lasting for 2-5 days. In addition, lack of response to high-dose antihistamines, corticosteroids or epinephrine is required[33]. Up to 75% of the patients have a family history of angioedema attacks. It is important to exclude that the patient is treated with angiotensin converting enzyme (ACE) inhibitors since angioedema may be a side effect. The onset of symptoms is

usually in childhood. HAEnCI have similar symptoms as HAE1 and 2 but in HAEnC1 the most common site of swelling is the face. HAEnCI is also more sensitive to estrogen, which makes women more vulnerable. One indicative of AAE is that the symptoms usually appear later in life, in patients aged 40 or above. The symptoms in AAE are similar to those in HAE1 and 2. There is no family history of angioedema in AAE.

Laboratory findings

HAE1 and 2 diagnoses must be verified by laboratory tests. Both types are characterized by low C4 levels. In HAE1, the C1INH protein level is decreased, usually below 30% of normal level. In HAE2 the level of C1INH is normal or increased, but the C1INH function is defective. If the level of C1INH is decreased or if the function of C1INH is defective, the test should be repeated to verify the findings. In children below 1 year of age, measurement of C1INH can be misleading since the level of C1INH is not stabilized until around one year of age[34]. Genetic testing is an option, with sequencing of the *SERPING1* gene. HAEnCI can to date only be verified genetically, but the mutations are very rare[33]. In AAE the C1INH level is decreased, as well as C4, but may be more variable than in HAE1 and 2. A characteristic feature of AAE is a decreased level of C1q in approximately 70% of patients[35]. Genetic testing gives no evidence of mutations affecting the *SERPING1* gene (Table 1). Patients with AAE should undergo testing for lymphoproliferative and autoimmune disease with physical examination, chest X-ray and abdominal ultrasound as well as laboratory testing for complete blood cell count with differential, serum protein electrophoresis and antinuclear antibodies (ANA)[35].

Table 1. Laboratory findings

	HAE 1	**HAE 2**	**HAEnCI**	**AAE**
C1INH level	↓	normal / ↑	normal	↓
C1INH function	↓	↓	normal	↓
C4 level	↓	↓	normal	↓
C1q level	normal	normal	normal	normal / ↓
Genetic analysis	*SERPING1*	*SERPING1*	*F12, ANGPT1, PLG, KNG1, MYOF, HS3ST6*	normal

Treatment

History

HAE is a potential life-threatening disease owing to the risk of asphyxia when edema involves the upper airways[36]. Before 1960, there were no treatments except for supportive care and attempts to keep the airways clear. By 1960, clinical trials supported androgenic male hormones as treatment[37]. Danazol was developed in 1963 and was reported as a treatment with good effect in HAE in 1976[38,39]. The mechanisms of action of attenuated androgens include induction of aminopeptidase P, a metallopeptidase involved in the degradation of bradykinin, and increased production of C1INH[40,41]. In the 1990s, oxandrolone, a synthetic anabolic steroid, became available for treatment. As a result of reports on side effects such as weight gain, virilization, menstrual irregularities, liver adenomas and serum lipid levels[42-44], attenuated androgens are no longer recommended as a first-hand choice of treatment. The antifibrinolytic drugs tranexamic acid and ε-aminocaproic acid were introduced in the mid-60s both as treatment for acute attacks and as prophylactic care. The effect is attributed to inhibition of the conversion of plasminogen to plasmin[45]. In 1970 three studies supporting the use of tranexamic acid were published[46-48]. Although new therapies are more effective, tranexamic acid is still in use, mainly in AAE where it has been shown to have a good effect, and in children[35]. Side effects include headache, nausea, and gastrointestinal symptoms. Fresh frozen plasma has been used as an acute treatment since late 1960s[49,50]. It is still in use in some countries without other treatment options. Fresh frozen plasma has gradually been replaced by plasma derived (pd) C1INH, which was approved for on demand treatment in 1986 in Germany, followed by approval in many countries[51]. Also, recombinant human (rh) C1INH concentrate, derived from the milk of transgenic rabbits is available. This has a shorter half-life than pd C1INH[52,53]. Pd C1INH is administered intravenously or subcutaneously, and many patients are trained to administrate it themselves. The mean plasma half-time of pd C1INH is longer than 30 hours. The tolerability and safety of pd C1INH is good, and only few adverse events have been reported. Treatment with plasma derived and recombinant human C1INH replaces the deficient or dysfunctional C1INH in HAE patients resulting in normalization of all cascade systems involved[33]. Since 2008, the bradykinin B2 receptor antagonist icatibant is available as an on demand treatment. It prevents vasodilatation, capillary leakage, and the development of subsequent angioedema. Icatibant is administered subcutaneously and is also approved for children[54]. Ecallantide, a kallikrein inhibitor for treatment of acute attacks, is available since 2009, only in the United States and a few Latin American countries[55]. Recently, long-term prophylaxes (LTP) targeting kallikrein have been developed; lanadelumab is a humanized monoclonal antibody inhibiting plasma kallikrein, thereby preventing the cleavage of high-molecular-weight kininogen generating

bradykinin[56]. It is administered subcutaneously every other week, and the treatment interval can be extended to several weeks if the patient is well controlled. Injection site reactions are reported as adverse events. Berotralstat is a small molecular compound inhibitor of plasma kallikrein, inhibiting its activity. It is administered orally at daily intervals. Gastrointestinal side effects are reported, although less frequent with time[57].

Emergency medication

On demand treatment is always considered upon episodes of angioedema, although involvement of the upper airways can make pharmacological intervention urgent. Gastrointestinal attacks with severe pain and pseudo-ileus may also warrant acute treatment. All patients are recommended to have access to on demand treatment at home. Attacks are to be treated during an early phase, before established angioedema, for best effect. Icatibant, ecallantide and intravenous C1INH are the recommended treatments of choice. The only on demand medication recommended so far during pregnancy is pd C1INH[33].

Short-term prophylaxis

Short-term prophylaxis may be indicated prior to situations where there is a risk of an attack, such as dental treatments, medical procedures, or situations of emotional stress. Intravenous pd C1INH is recommended as first-line short-term prophylaxis and should be administered 1 to 12 hours before the procedure. Androgens can also be used 5 days before and 2-3 days after the procedure[51,33].

Long-term prophylaxis

Medications that prevent attacks can reduce the burden of the disease. Patients with frequent and/or severe attacks, may be subject to LTP. Substitution with pd C1INH is the most physiological approach to treat HAE. It has been approved and used as LTP since 2008 and is now also approved for children. It is usually administered twice weekly, intravenously or subcutaneously. Generally, pd C1INH, lanadelumab and berotralstat are recommended as first-line LTP treatment. Attenuated androgens have been used as LTP for decades, although concerns are raised regarding side effects, such as weight gain, hair loss and menstrual irregularities. Regular monitoring of liver function and lipid levels should be performed. Attenuated androgens are therefore recommended as second-line treatment[58,59]. Tranexamic acid is mainly used in females and children.

Emerging treatment modalities

Treatment of bradykinin-mediated angioedema has been subject to extensive research the last decade which has resulted in multiple novel therapy options, both for acute attacks and as prophylaxis. During the last five years, 16 randomized trials regarding HAE treatments were published; seven involved C1INH concentrates, seven investigated kallikrein inhibitors, one studied prekallikrein inhibitor, and one investigated Factor (F) XIIa inhibitor[60,61]. Although FXII is the initiator of the intrinsic coagulation cascade leading to fibrin formation, FXII deficient persons have normal hemostasis and do not exhibit a clinically relevant bleeding phenotype[62].

The following targets and pharmacological substances are currently under development:

Long-term prophylaxis

Targeting FXIIa:

- Garadacimab - a humanized anti-FXIIa monoclonal antibody, administered subcutaneously every 4 weeks (200 mg)[61] (phase III trial).
- ALN-F12 - a small interfering RNA to down-regulate expression of FXII, administered subcutaneously[63] (preclinical studies).
- KV998086 - a small molecular compound, administered orally (preclinical studies).

Targeting prekallikrein:

- Donidalorsen - an antisense oligonucleotide, administered subcutaneously every 4 weeks[64] (phase III trial).
- NTLA-2002 works by CRISPR/Cas 9 gene editing delivered via lipid nanoparticles, administered intravenously once (phase I-II trials).

Targeting kallikrein:

- STAR-0215 - a monoclonal antibody, administered subcutaneously every 3 months or longer (phase I-II trials).
- ATN-249 - a small molecular compound, administered orally (phase I trial).

Targeting C1INH:

- BMN 331 is based on adeno-associated virus (AAV)5 gene therapy, administered intravenously once. It induces an extra-chromosomal copy of *SERPING1* gene enabling individuals to produce their own functional C1INH protein (preclinical – phase I trial).

- OTL-105 - an *ex vivo* autologous hematopoietic stem cell gene therapy in order to increase C1INH production, administered intravenously once (preclinical studies).

Targeting the bradykinin B2 receptor:

- PHVS719 - a small molecular compound, administered orally (extended-release tablet) (phase I trial).
- PHVS416 - a small molecular compound, administered orally (phase II trial).

Acute therapy

Targeting kallikrein:

- Sebetralstat (KVD 900) - a small molecular compound, administered orally (phase III trial)[65].

Targeting the bradykinin B2 receptor:

- PHVS416 - a small molecular compound, administered orally (phase II trial).

The immune system

The immune system is classically divided into the innate and the adaptive immune system. The innate immune system is the early defence, responding fast, within hours and in the same way to invading pathogens, but it also instructs the adaptive immune system how to respond most effectively to different microbes. The adaptive immune system has a memory and is specific to enable response to a particular pathogen. The adaptive immune response requires longer time, usually 4-7 days, to produce antibodies (Figure 1).

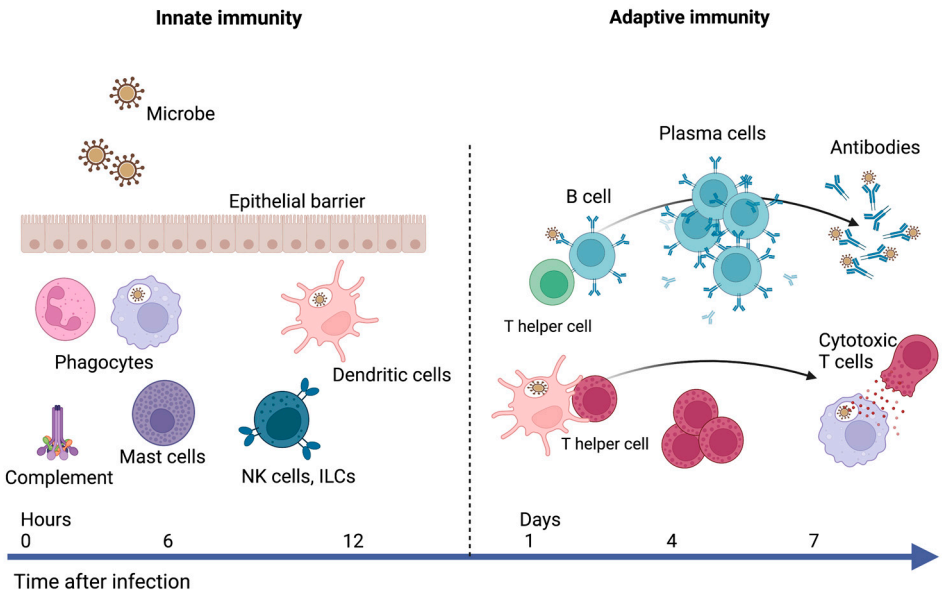

Figure 1. Innate and adaptive immunity. Created with BioRender.com

Innate immunity

The innate immune system consists of epithelial barriers, phagocytes, mast cells, dendritic cells, the complement system, innate lymphoid cells (ILCs) and natural killer (NK) cells, all of which are close to the epithelial barrier, where pathogens first encounter the body.

Barriers (epithelium)

The major entry routes of microbes; the skin, respiratory-, gastrointestinal- and urogenital tract are lined by epithelial barriers that protect the body from intruding

pathogens. Epithelial cells play important roles in host defence through inflammation and regulation of immune responses by release of cytokines and chemokines. Furthermore, cilia and mucus aid in the removal of pathogens. Antimicrobial peptides, for example lysozyme, produced by epithelial cells serve as innate antibiotics[66].

Macrophages

Macrophages are present in all tissues and body compartments with an overall function of phagocytosis and antigen-presentation. Macrophages also release cytokines and other mediators to recruit other inflammatory cells, such as neutrophils and T cells to the sites of inflammation[67,68].

Granulocytes

Granulocytes are divided into neutrophils, eosinophils, and basophils. Neutrophils are important players during acute inflammation. They have different mechanisms to eliminate pathogens, such as phagocytosis, degranulation, and formation of neutrophil extracellular traps (NETs). Eosinophils and basophils are activated and recruited to tissues by T helper type 2 (Th2) cells predominantly in responses to parasite infections as well as during allergy and asthma. At the site of infection, eosinophils release granules containing cytotoxic proteins, cytokines and other mediators giving rise to inflammation[69,70].

Mast cells

Mast cells are resident in tissues near blood vessels, nerves, mucosal and epithelial tissues. They are predominantly involved in immune responses against parasites, but also in allergic reactions, where they release preformed proinflammatory mediators when activated by allergens binding to and crosslinking IgE antibodies bound to the mast cell surface. Mast cells can also respond to products of complement activation and other substances[71].

Dendritic cells

Dendritic cells reside predominantly in the skin, airways, and gastrointestinal tract where microbes can invade tissues. They are a link to the adaptive immunity by presenting antigens to T cells in the peripheral lymphoid tissues after uptake and processing. They also produce cytokines that contribute to the recruitment of leukocytes[72].

The complement system

The complement system consists of more than 40 proteins which are activated in a sequential enzymatic cascade ultimately leading to inflammation, opsonization, phagocytosis, and lysis of the pathogen. The complement system can be activated

by three different pathways; the classical, the lectin and the alternative pathway (Figure 2). The complement system will be described in detail later.

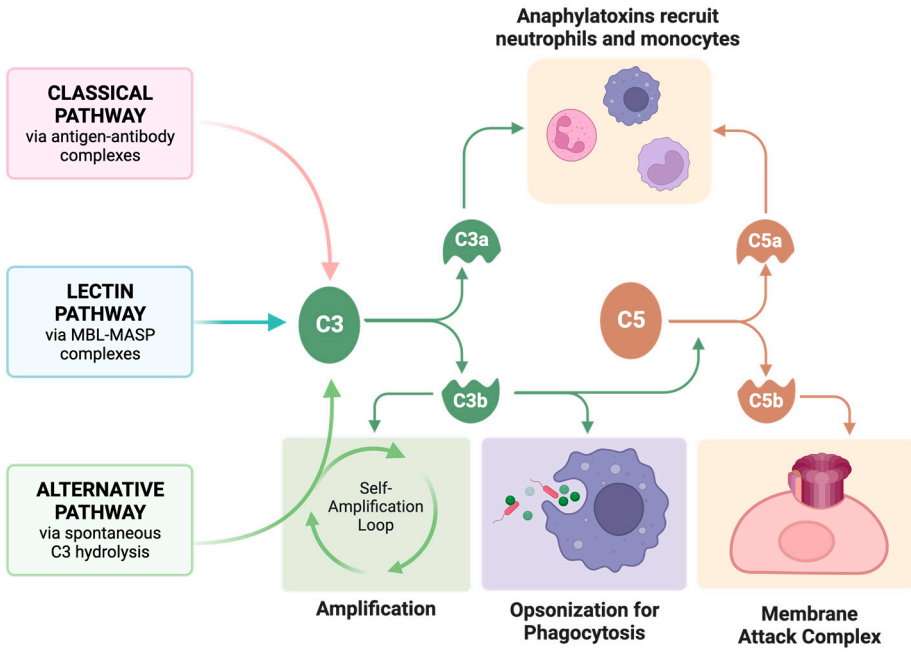

Figure 2. The complement system. Reprinted from BioRender.com.

Innate lymphoid cells

Innate lymphoid cells (ILCs) are lymphocytes mainly residing in mucosal surfaces. They lack specific antigen receptors and are the innate counterparts of T lymphocytes. ILC1s, like Th1cells, mainly react to intracellular pathogens such as viruses and tumours. ILC2s, like Th2 cells, respond to allergens and parasites, and ILC3s, like Th17 cells, react to extracellular microbes such as bacteria and fungi[73].

NK cells

Natural killer (NK) cells are lymphocytes in the innate immune system and belong to group 1 of the innate lymphoid cells (ILC1). NK cells are an important first-line defence against viral infections and have cytotoxic capacity to kill virally infected cells as well as tumour cells by lysis. NK cells produce inflammatory cytokines, predominantly IFN-γ and TNF-α which promote phagocytosis and activate antigen presenting cells (APCs). NK cells also have the capacity to regulate T cell responses, and there is increasing evidence that NK cells are of great importance for protection against autoimmunity[74-76].

Adaptive immunity

Lymphocytes are key mediators of adaptive immunity with the ability to produce specific receptors for antigens. Lymphocytes are primarily divided into T cells and B cells, displaying different ways to eliminate microbes. All lymphocytes derive from stem cells in the bone marrow. T cells mature in the thymus and B cells in the bone marrow. The lymphocytes then enter the circulation and the peripheral lymphoid organs, consisting of the lymph nodes, the spleen, the tonsils and the mucosal immune system, where they may be activated by antigens. Activated lymphocytes then enter the bloodstream and migrate to the site of infection. An important aspect characterizing adaptive immunity is immunological memory, which means that the immune system responds faster and more effectively to repeat encounters with the same antigen[77].

T cells

T lymphocytes belong to the category of cell mediated immunity. T cell receptors (TCRs) recognize fragments of antigens presented by human leukocyte antigen (HLA) molecules, also called major histocompatibility complex (MHC) class I and class II on APCs. T cells can broadly be divided into helper T cells ($CD4^+$), and cytotoxic T cells ($CD8^+$). MHC class I molecules, present on all nucleated cells, display peptides from intracellular pathogens such as viruses and endogenous peptides, and are recognized by $CD8^+$ T cells. MHC class II display peptides derived from endocytosed proteins, such as extracellular microbes and are restricted to dendritic cells (DCs), macrophages and B lymphocytes, recognized by $CD4^+$ T cells. In order to be fully activated, T cells need a second signal, a co-stimulator called B7 protein on APCs recognized by a receptor called CD28 on T cells[78]. $CD4^+$ T cells can further be divided into T helper (Th)1, Th2, Th17 and T follicular helper (fh) cells, as well as additional emerging $CD4^+$ T cell subsets. Th1 cells play a major role in clearance of intracellular pathogens like viruses and intracellular bacteria by activating $CD8^+$ T cells to become cytotoxic T cells, activation of macrophages and production of interleukin (IL)-2, IFN-γ and TNF-α. Th2 cells produce IL-4, IL-5, IL-9, and IL-13 which facilitate B cell proliferation and antibody response. IL-4 and IL-5 drive IgE production and eosinophilic inflammation, so called type 2 inflammation, playing an important role in the defence against parasites as well as in allergic response. Th2 cells are main actors in the development of severe asthma[79]. Th17 cells are involved in the defence against extracellular pathogens, recruitment of neutrophils and production of inflammatory cytokines like IL-17, IL-6, and TNF-α. Another category of $CD4^+$ T cells is called regulatory T cells (T regs), with the ability to inhibit T cell proliferation and cytokine production. T regs play an important role in preventing autoimmunity[80]. $CD8^+$ T cells are called cytolytic because they have the capacity to lyse cells harbouring intracellular microbes.

After an infection, a fraction of activated T cells differentiates into long-lived, functionally inactive memory cells (both $CD8^+$ and $CD4^+$). Memory T cells are

essential to maintain immunological memory in order to protect against future exposure of the same pathogen. They circulate for months or years and are divided into central and effector memory cells. Central memory T cells reside in lymphoid tissues, with the potential to divide rapidly and differentiate to effector cells in response to antigen. Effector memory T cells have effector functions and circulate to peripheral tissues[81-83].

B cells

B cells recognize antigens via membrane bound antibodies on their surface that serve as receptors. If the antigen is a protein, it is internalized, processed, and presented by MHC class II in the peripheral lymphoid tissues. In order to produce antibodies, B cells need to interact with T helper cells specific for the same antigen. The T cell receptors (TCRs) on $CD4^+$ T cells recognize fragments of the antigen on MHC class II on B cells and activate B cells by expressing the costimulator CD40 ligand (CD40L) and secreting cytokines. CD40L binds to CD40 expressed on B cells. The activation of B cells leads to clonal expansion of antigen-specific B cells and differentiation into plasma cells that produce and secrete antibodies[84] (Figure 3). Other B cell activating factors are the recognition of microbial products by Toll-like receptors (TLRs), and complement factors bound to microbes. There are five classes of antibodies; immunoglobulin (Ig) G, IgA, IgM, IgD and IgE. B cells have immunological memory and recall previous contact with antigens, which leads to a quicker and more efficient subsequent response.

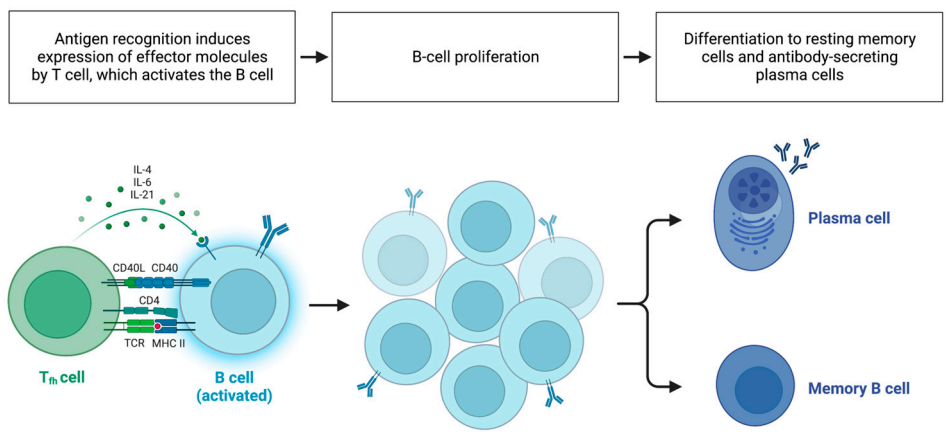

Figure 3. Steps in B cell differentiation. Reprinted from BioRender.com

Immunological tolerance

The immune system has different mechanisms to distinguish between harmful antigens and self-antigens to prevent self-destructive responses. Failure of these

mechanisms may result in attacks on the individual´s own cells and tissues by the immune system, known as autoimmunity.

Central T cell tolerance develops in the thymus. Positive selection occurs when the TCR of double-positive (expressing both $CD4^+$ and $CD8^+$) T cells bind with low avidity to MHC molecules displaying self-antigens. The T cells that do not bind are eliminated. In the second step called negative selection, the T cells with a strong binding to self-antigens bound to MHC molecules are deleted. This selection is accomplished by medullary thymic epithelial cells (mTECs) and bone marrow derived dendritic cells interacting with T cells. The mTECs express a transcription factor called the autoimmune regulator (AIRE) and are able to display tissue-restricted proteins to T cells. Many self-reactive T cells escape this negative selection and enter the periphery[85,86].

Peripheral tolerance is a mechanism that takes place in the peripheral lymphoid tissues leading to inactivation, so called anergy, or death of the mature T cells encountering self-antigens without adequate second signal (B7). At high level of TCR self-reactivity, expression of cytotoxic T lymphocyte associated protein 4 (CTLA-4) is induced on APCs and competes with CD28 for ligation with B7 molecules, inhibiting T cell activation. Variants in the *CTLA4* gene are associated to thyroid autoimmunity and diabetes type 1. Another mechanism of peripheral tolerance is performed by regulatory T cells (T regs), a subset of self-reactive $CD4^+$ T cells dependent on the transcription factor Foxp3. Their role is to suppress self-reactivity and promote tissue repair. A deficiency of T regs is proposed to be involved in autoimmune disease[87].

Central B cell tolerance takes place in the bone marrow, where immature B cells recognizing self-antigens either undergo apoptosis or change their receptor specificity, called receptor editing[87]. However, a significant fraction of autoreactive B cells enters the periphery. The selection of B cells into the mature compartment occurs via transitional B cells. Autoimmunity has been associated with a relative expansion of transitional cells, which may reflect or predispose to loss of B cell tolerance[88]. Peripheral tolerance of B cells develops when the B cell encounters an antigen without the help of a T cell, and the B cell becomes anergic. B cells can also be excluded from lymphoid follicles and undergo apoptosis if the activation is partial. Signal two from T helper cells responding to a foreign antigen might be misdirected to self-reactive BCRs cross-reacting with a component of a microorganism, as in Guillain-Barré syndrome, where antigens from Campylobacter jejuni cross-react with components of peripheral nerves leading to production of autoantibodies. Another pathway for antibody production in B cells is by TLR signaling, initiated by microorganisms, without the help of T cells. A dysregulation in this pathway may drive the production of autoantibodies[89-91].

C1 inhibitor

C1inhibitor (C1INH) was discovered in 1957, described to inhibit the activity of the first component of complement (C1), hence its name[92,93]. C1 inhibitor, also known as C1 esterase inhibitor, belongs to the serpin (Serine Protease Inhibitor) superfamily. There are 37 human serpins, regulating the activity of proteases involved in physiological processes such as inflammation, hemostasis, tissue remodeling and angiogenesis[94]. Proteases operate in cascade activations and need to be controlled by inhibitors (serpins). In humans, loss of serpins can have severe pathological consequences, including emphysema caused by alpha-1 antitrypsin deficiency, thrombosis caused by antithrombin deficiency and angioedema caused by C1INH deficiency[95]. C1INH is made up of three β-sheets and nine α-helices. The region responsible for interaction with proteases, the reactive center loop (RCL), is exposed on the surface of the molecule and incorporated into one of the β-sheets[96]. After docking of the protease to C1INH the protease cleaves the RCL, which leads to a conformational change within the serpin molecule. This change largely increases the stability of the molecule. During this process, the protease active site becomes distorted[96]. The serpin-protease complex will then be cleared via scavenger receptors[94,97].

C1INH inactivates proteases in the complement system (C1r, C1s, MASP1 and MASP2), the contact activation system (FXIIa and plasma kallikrein), the intrinsic coagulation system (FXIIa and FXIa) and the fibrinolytic system (plasmin and tissue plasminogen activator (tPA))[93] (Figure 4).

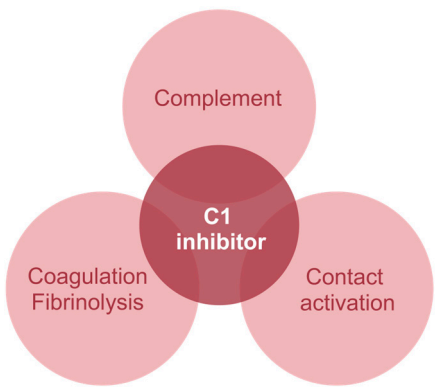

Figure 4. The interactions of C1 inhibitor

Complement system

The complement system plays an important role in the defence against invading pathogens and damaged cells. It consists of proteins that are activated in a cascade system[98-100]. The complement system is activated *via* three pathways; the classical, the lectin and the alternative pathway. The classical pathway is triggered by antibody-antigen complexes and the lectin pathway is initiated by recognition of carbohydrate structures by MBL, ficolins and collectins. The alternative pathway is activated by damaged or foreign cells, certain microbial compounds or the spontaneous low-grade hydrolyzation of C3[101,102]. Activation of the classical pathway leads to cleavage of C4 to C4a and C4b, and subsequently cleavage of C2 to C2a and C2b. C4b complexes with C2b to form an enzyme complex called C3 convertase (C4bC2b) which cleaves C3 into the anaphylatoxin C3a, and C3b. Activation of the lectin pathway leads in a similar way to cleavage of C2 and C4 resulting in a C3 convertase. C3b becomes attached to a microbe or other activating surface, facilitating opsonisation. Some of the C3b binds to the C4bC2b complex, and the resultant complex (C4b/2b/C3b) functions as a C5-cleaving enzyme, a C5 convertase. Factor B binds to C3b and is cleaved by Factor D, resulting in Ba and another C3 convertase; the C3bBb complex. The alternative pathway provides an amplification loop by enhancing C3 activation by properdin, generating more C3b and C3bBb complexes. C3b binds to the C3bBb complex to form another form of C5 convertase (C3bBbC3b). The two forms of C5 convertase cleave C5 into the anaphylatoxin C5a, and C5b. Generation of C5b is the start of the terminal pathway which leads to the formation of the membrane attack complex (MAC) which may lead to lysis of bacteria and cells when inserted into a cell membrane[98]. Activation of the complement system also generates several biologically relevant components like C3-, C5- and C4-derived fragments that induce inflammatory reactions and stimulate adaptive immune cells[103].

The first component of the classical pathway is the C1 complex. It consists of C1q, which is a pattern recognition molecule, and four serine proteases; two molecules of C1r and two molecules of C1s. C1q in the C1 complex recognizes and binds to antibodies bound to an antigen on the surface of for example a bacteria, which trigger autoactivation of C1r. Activated C1r subsequently cleaves C1s, which in turn cleaves C4 and C2 forming a C3 convertase. The active sites of the C1r molecule are facing outwards, which make them easily accessible to C1s as well as to C1INH. Both activated C1r and C1s can bind to and react with C1INH, forming complexes which dissociate from C1q, thereby preventing further activation of the classical pathway[104,105]. C1INH is the only plasma protease inhibitor that regulates the classical pathway.

The lectin pathway consists of MBL, ficolins or collectins and the serine proteases MASP1 and MASP2, in a complex similar to the C1 complex. The lectin pathway is initiated by recognition and binding of the complex to carbohydrates on a

microbial surface. MASP2 is the key enzyme of the lectin pathway which then autoactivates cleaving C4 and C2 to form a C3 convertase. MASP2 can also activate prothrombin, leading to generation of thrombin and via subsequent action of thrombin, to formation of cross-linked fibrin by cleavage of fibrinogen to fibrin, and activation of FXIII[106,107]. MASP1 cleaves C2 to a lesser extent than MASP2 and may cleave C3 directly. MASP1 has also other functions outside the complement system. C1INH is a regulator in the lectin pathway, by inactivation of MASP1 and MASP2, thereby inhibiting further activation of the lectin pathway. α-2 macroglobulin also has the ability to inhibit MASP2, although a weaker inhibitor than C1INH[108,93] (Figure 5).

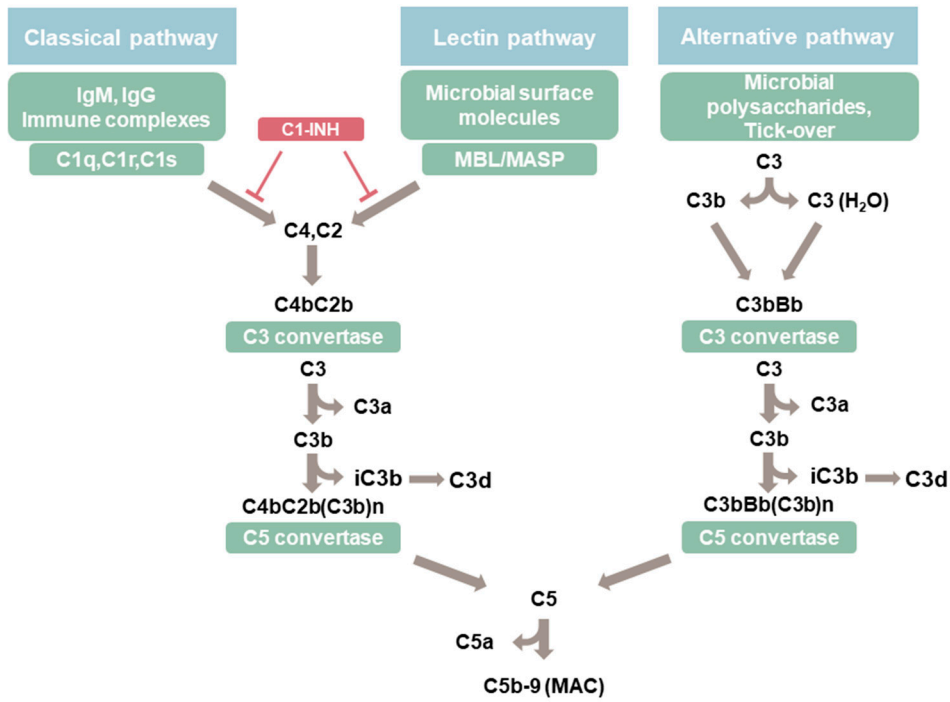

Figure 5. The complement system including the sites where C1INH is involved.

Contact activation system

The contact activation system (CAS) is often considered synonymous with the plasma kallikrein/kinin system, although they are two overlapping and interacting systems. Both systems have main roles in inflammation, and the CAS is also involved in thrombin formation[109]. The CAS is activated when the endothelial cell surface is exposed to initiating factors (negatively charged proteins) that activates FXII to form

FXIIa. FXIIa activates prekallikrein to form kallikrein, which in a reciprocal way activates FXII to FXIIa. Kallikrein cleaves high molecular weight kininogen (HK), producing bradykinin. Bradykinin is a nonapeptide, particularly important in blood pressure regulation and in inflammatory reactions. Bradykinin degrades very fast, with a half-life of about 30 seconds. Degradation is primarily mediated by angiotensin converting enzyme and by plasma carboxypeptidase N[110,111]. Bradykinin and lys-bradykinin mediate their activity through binding of the B2 receptor and the B1 receptor, two G-protein-coupled receptors, leading to vascular permeability and edema. The B2 receptor is constantly present on the vascular epithelium and the B1 receptor is expressed transiently at sites of inflammation[94]. The major inhibitor of kallikrein and FXIIa is C1INH (Figure 6).

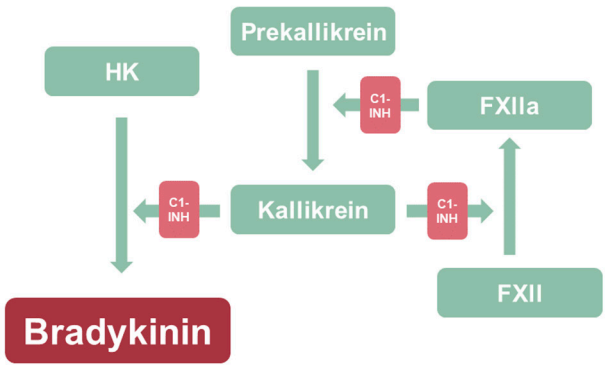

Figure 6. Contact activation system including the sites where C1INH is involved.

Coagulation system

The coagulation cascade can be subdivided into three pathways; the extrinsic pathway, the intrinsic pathway and the common pathway. The extrinsic pathway starts at the site of an injury, when blood is exposed to tissue factor (TF) expressed in the subendothelial tissue. TF binds factor VIIa and calcium to promote the conversion of FX to FXa. The TF-FVIIa complex also activates FIX to FIXa[112-114]. The intrinsic pathway starts with activation of FXII to FXIIa upon exposure to negatively charged surfaces or pathogens. FXIIa activates FXI to FXIa which further activates FIX to FIXa forming a complex with FVIIIa to activate FX. The final event of the coagulation cascade, where the extrinsic and the intrinsic pathways converge, is when FXa along with the cofactors FVa and calcium form a complex which converts prothrombin to thrombin. Thrombin further cleaves fibrinogen to fibrin which incorporates and stabilizes the platelet plug[115,116]. The coagulation pathway is downregulated in vivo by C1INH, which is the only significant inhibitor of FXIIa and FXIa[114,117,110] (Figure 7).

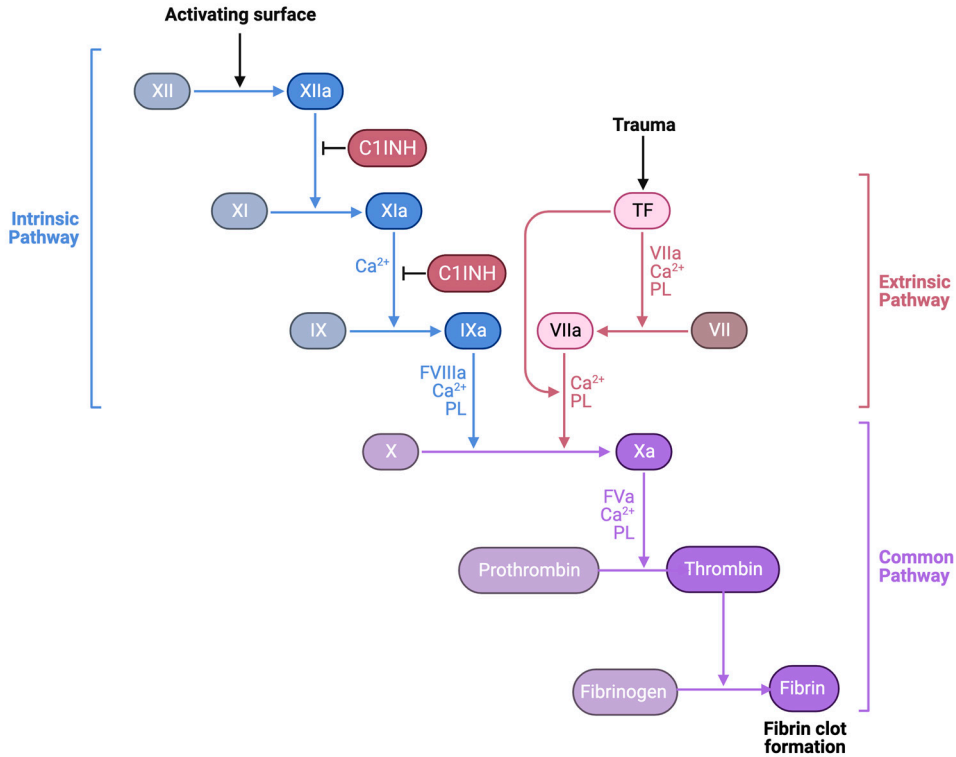

Figure 7. The coagulation cascade including the sites where C1INH is involved. Created with BioRender.com

Fibrinolytic system

Fibrinolysis is the physiological breakdown of fibrin with the purpose of limiting and dissolving blood clots. Fibrin is primarily degraded by plasmin. Plasminogen can be converted to plasmin by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). Plasmin activity is regulated by its inhibitor alpha-2 antiplasmin preventing widespread fibrinolysis[113]. Plasminogen activator inhibitors 1 and 2 inhibit tPA and uPA[118,119]. Plasmin is considered a natural trigger for production of bradykinin[11]. Bradykinin itself stimulates the release of tPA from endothelial cells, thereby forming plasmin. Plasmin can also modulate complement activation generating anaphylatoxins C3a and C5a[120]. C1INH inactivates plasmin and tPA[93]. Antifibrinolytic agents such as tranexamic acid and ε-aminocaproic acid are able to reduce the severity and frequency of HAE attacks by binding to specific lysine binding sites on plasminogen, reducing the generation of plasmin. The therapeutic effect supports that plasminogen activation has a role in HAE pathogenesis[120,121]. D-dimers are fibrin degradation products that reflect in vivo

activity of the fibrinolytic system and are specific indicators of fibrinolysis used in the diagnosis of venous thromboembolism[113].

Thromboembolic disease

Hemostasis is a balance between procoagulant and anticoagulant systems. Procoagulant systems include platelets and the coagulation cascade. Anticoagulant systems consist of activated protein C/protein S, fibrinolysis and serpins. If there is a defect in one of these systems, hemostasis is out of balance which may result in either thrombosis or bleeding. Virchow´s triad describes the important categories of factors that contribute to thrombosis; hypercoagulability, stasis of blood flow and endothelial injury[121,122].

Arterial thromboembolism

Arterial thrombi are mainly composed of aggregated platelets and are usually the result of rupture of an atherosclerotic plaque. Arterial thromboembolism (ATE) can cause ischemic injuries of which the most severe clinical manifestations are cardiac ischaemia and stroke. Risk factors for cardiovascular disease are hyperlipidemia, smoking, diabetes, hypertension, and abdominal obesity. Several epidemiological studies also show associations between obesity, metabolic syndrome, type 2 diabetes and VTE[123,124].

Venous thromboembolism

In venous thrombosis, the endothelium remains intact. The thrombi are mainly composed of fibrin and red cells. Venous thrombosis can be triggered by abnormal blood flow, altered properties of the blood and endothelial alterations. Venous thromboembolism (VTE) is the most common vascular disease after acute myocardial infarction and stroke. The main clinical manifestations are deep venous thrombosis (DVT) and pulmonary embolism (PE). The underlying pathogenesis is still not completely known[125,126].

Risk factors

Strong risk factors for VTE are trauma/fractures and major surgery. Moderate risk factors are surgery, malignancy, oral contraceptives, pregnancy, and puerperium [127]. Weak risk factors are increasing age, bed rest > 3 days, prolonged travel and metabolic syndrome. Some coagulation factors increase with age; factors V, VII, VIII, IX, fibrinogen, and tissue factor[128]. The risk of VTE is also increased due to inherited deficiencies of natural inhibitors of coagulation factors such as

antithrombin, protein C and protein S. These deficiencies have little or no effect on arterial thrombosis. The two most common genetic risk factors for VTE are a mutation in the factor V gene (factor V Leiden) and a mutation in the prothrombin gene. These two mutations also increase the risk of ATE, but to a lesser extent[129].

Antiphospholipid syndrome

One of the most important acquired risk factors associated with both arterial and venous thrombosis, including pregnancy complications is the antiphospholipid syndrome (APS). The diagnosis of APS is defined as the combination of circulating antiphospholipid antibodies and a history of thrombosis and/or pregnancy morbidity including fetal loss. The antiphospholipid antibodies include anti-beta2-glycoprotein I, anti-cardiolipin and lupus anticoagulant antibodies. These antibodies are directed against cofactors on phospholipid membrane surfaces that lead to interactions with cells resulting in activation of platelet and coagulation pathways[130].

Autoimmune disease

Autoimmune diseases (ADs) affect around 5% of the world population. ADs are conditions in which immune responses to self-antigens lead to disease development. There is a great variation in the clinical manifestations, some being limited to a particular organ and some being systemic. ADs are thought to arise from a combination of genetic and environmental factors[131].

Mechanisms

Genetic predisposition

The strongest genetic predisposition to autoimmune responses, regardless of underlying cause of autoimmunity is associated with certain HLA alleles[132]. Cytokine and cytokine receptor polymorphisms are linked to ADs such as Behcet´s disease, Crohn´s disease and psoriasis[133]. There are examples of ADs which result from a single genetic mutation, such as autoimmune polyendocrine syndrome resulting from a mutation in the *AIRE* gene[134]. Another example of a monogenic disorder is homozygous C1q deficiency, leading to SLE.

Hormonal factors

Women are more affected by ADs than men. Approximately 80% of patients with an autoimmune disease are women. Estrogen affects the survival and selection of

autoreactive B cells, leading to increased autoantibody production. Female gender is also associated with polyautoimmunity[135-137].

Environmental triggers

Antigen mimicry, a process in which the immune system reacts to self-antigens similar to molecules on the invading pathogen, so called cross-reaction, is a major trigger of autoimmune disease[131]. Another environmental trigger is UV irradiation leading to cutaneous lupus. This could be explained by induction of apoptotic cell death by UV radiation leading to an increased exposure of nuclear antigens to the adaptive immune system[138]. A dysregulated immune response to the gut microbiome is discussed as a possible cause of some autoimmune diseases such as inflammatory bowel disease and type 1 diabetes. Smoking and other toxic factors can also affect induction of ADs[139].

Defective regulation

Failure of self-tolerance due to defects in the regulation of B cells, imbalance between effector T cells and Tregs, and altered peptide/MHC-recognition are proposed factors of defective regulation of the immune response.

Manifestations of autoimmune disease

SLE

Systemic lupus erythematosus (SLE) is a chronic inflammatory AD primarily affecting women of childbearing age, with a female to male ratio of 9:1[140]. The prevalence is 68/100,000 in Sweden. The diagnosis is based on clinical manifestations combined with certain autoantibodies. More than one organ is usually involved, such as the skin, kidneys, and joints. The pathogenesis includes production of autoantibodies and formation of immune complexes that can be deposited and ultimately damage organs[141,142]. Common symptoms are fatigue, fever, joint- and muscle ache, skin rash and mouth ulcers. Many ADs, such as SLE, are associated with an increased risk of cardiovascular complications, including VTE. Some studies demonstrate a three- to six-fold higher risk of VTE in patients with SLE compared with the general population. A considerably higher risk is seen in SLE patients with anti-phospholipid syndrome (APS) compared to SLE patients without APS. Common features in ADs including SLE are inflammation and hypercoagulability, two factors that are responsible for inciting VTEs. It has been suggested that the production of autoantibodies is caused by inefficient removal of cellular components during cell death, leading to prolonged exposure of autoantigens (e.g. nuclear proteins and DNA) with subsequent presentation to T cells, leading to production of autoantibodies. This is called the waste - disposal

theory[143-145]. There is also a prominent dysregulation of interferon in SLE, which plays a major role in the pathogenesis[146].

Comorbidities in HAE

Complement and autoimmune disease

The complement system is involved in the pathogenesis of several autoimmune diseases, such as SLE, vasculitis, Sjögren´s syndrome, APS and RA[147]. Links between the complement system and SLE were first identified when it was discovered that complement levels were decreased in these patients. Subsequent clinical observations have shown a strong connection between SLE and deficiencies of the early part of the classical pathway. More than 90% of individuals with C1q and C1r/C1s deficiency develop an SLE-like disorder[148]. Patients with homozygous deficiencies of C4 also have a high prevalence of SLE-like disease[140,149,150].

In SLE, complement activation via the classical pathway is thought to be triggered by the formation of immune complexes, leading to a consumption of complement proteins. Tissue damage is mainly mediated by the anaphylatoxins C3a and C5a, and the membrane attack complex (MAC)[151].

The mechanisms that associate development of SLE with complement deficiency, particularly in the classical pathway, are not clear but there are some main hypotheses. The complement receptor CR1 on erythrocytes are important in the removal of immune complex from the circulation. In complement deficiency there is a lower level of CR1, leading to more circulating immune complexes which can be deposited in tissues[148,152]. Another explanation is the waste-disposal theory, according to which clearance of apoptotic cells and immune complexes is defect, resulting in exposure of autoantigens that drive the production of autoantibodies and tissue inflammation[140]. Complement components also have a role in regulation of cytokine production, and complement deficiency might lead to impaired cytokine production, such as type 1 interferons that are shown to have a role in SLE[148].

Complement and autoimmune disease in HAE

In HAE, the theory behind the increased risk of ADs in general and SLE in particular, is thought to be increased activation of the classical and lectin pathway due to lack of C1INH, which leads to increased consumption of C4 resulting in a reduction of C4 and C2 which, in the same way as primary deficiency of early components of the complement system, gives rise to SLE-like disorders.

Previous studies have presented an increased risk of autoimmune disease in patients with HAE[153-155]. There are especially several reports on SLE in patients with HAE[156-160], but also those describing thyroid autoimmunity[161], Crohn´s disease[162],

rheumatoid arthritis[163] and glomerulonephritis[164,165]. Also, elevated levels of autoantibodies are shown in HAE patients, with as much as nearly 50% of patients positive for autoantibodies in some studies[166,167]. There is evidence of lower capacity for opsonization and immune complex solubilization in serum from patients with HAE compared to serum from healthy controls[168]. Taken together, there is a clear propensity to produce autoantibodies and to develop autoimmune diseases in HAE patients.

Lymphocytes in HAE

Very little is published regarding lymphocytes and other immune cells in HAE patients. In a previous study, a total T cell count was increased in HAE patients, as well as an increased number of T helper cells[169]. In a study investigating 61 HAE patients, increased B cell activation was found[170]. There are many studies on lymphocytes in SLE which is interesting since SLE is the most common AD in HAE. There is increasing evidence that NK cells are involved in SLE pathogenesis, which is presented in several studies. A reduced number of NK cells in peripheral blood has been shown in several studies in SLE patients as well as in rheumatoid arthritis, diabetes type 1, autoimmune liver disease and psoriasis[171,172,76]. In an SLE mouse model, adoptive transfer of NK cells delayed the onset of autoimmunity, suggesting a protective role for NK cells in SLE[173,174].

Thromboembolism and HAE

C1INH is a multifunctional serine protease inhibitor, also inhibiting the intrinsic coagulation pathway, targeting kallikrein, FXIIa and FXIa. Consistent with this, significantly increased levels of the coagulation markers prothrombin fragment 1 + 2, D-dimer and thrombin-antithrombin (TAT) complexes have been demonstrated in patients with HAE compared to controls, both in remission and even further increased during attacks[175-177]. Another finding in HAE patients is shortened activated partial thromboplastin time (APTT) which together with elevated D-dimer and TAT complexes are previously shown to be associated with an increased risk of VTE[178,179]. An inverse correlation between C1INH and thrombin and prothrombin fragment 1 + 2 supports the involvement of coagulation activation in the pathophysiology of HAE[180]. C1INH is also an inhibitor of MASP1 and 2 in the lectin pathway of the complement system. Interestingly, it is shown that high levels of MASP2 are associated with an increased risk of VTE[181].

Plasma derived C1INH is used both as on demand treatment and as long-term prophylaxis. Reports indicate that patients treated with pd C1INH as prophylaxis had lower levels of prothrombin fragment 1+2 and D-dimer compared to patients receiving placebo[182], and a reduced incidence of VTE in HAE patients treated with C1INH compared to untreated HAE patients[183].

Recently, studies have been conducted to evaluate the impact of C1INH deficiency on coagulation and thrombosis using plasma from HAE patients and C1INH-deficient mice. These results showed an increase in contact pathway-mediated coagulation in both human and mice and an enhancement of venous thrombosis in C1INH deficient mice[184].

Aims

In addition to controlling the activation of the classical and lectin pathway of complement, C1INH also regulates several pathways of the contact system, coagulation system, and fibrinolytic system. As a result, low levels or defective function of C1INH may result in comorbid conditions, in addition to the characteristic episodes of angioedema. The overall aim of this thesis was to study the extent of possible comorbidities in patients with HAE, and if there are alterations in the adaptive immune system in peripheral blood of HAE patients that may explain a vulnerability to develop such comorbidities. We also wanted to investigate the trend in treatments of HAE as these may have bearing on the development of comorbid conditions.

The specific aims were as follows:

Paper I	To investigate if HAE type 1 and 2 modify the risk of contracting other diseases.
Paper II	To investigate if there is an increased risk of venous thromboembolism in HAE patients.
Paper III	To study trends in medications in HAE, both regarding disease-specific drugs as well as the use of drugs that may interfere with the clinical control of HAE.
Paper IV	To study lymphocyte populations and complement in relation to disease activity in patients with HAE.

Patients and methods

Paper I, II and III

Paper I, II and III are retrospective register-based studies evaluating the prevalence and cumulative incidence of comorbidities in patients with HAE compared to a control cohort from the general population in Sweden. By personal contact with all medical doctors identified via the national Swedish HAE medical network treating patients with hereditary angioedema, as well as medical doctors at clinical immunology laboratories in Sweden, we were able to identify 216 individuals with HAE1 and 23 individuals with HAE2, thus a total of 239 individuals who made up the patient cohort. All HAE diagnoses were based on a history of angioedema, low C4 concentrations in combination with low levels or defective function of C1INH. Patients with HAEnCI were not included in this study. With help from Statistics Sweden (SCB) 10 controls per patient were identified, matched for age, sex, and county of residence. For one patient only 3 matched controls were identified, hence the control cohort included 2 383 individuals. These two cohorts were compared in the following Swedish registers (Figure 8):

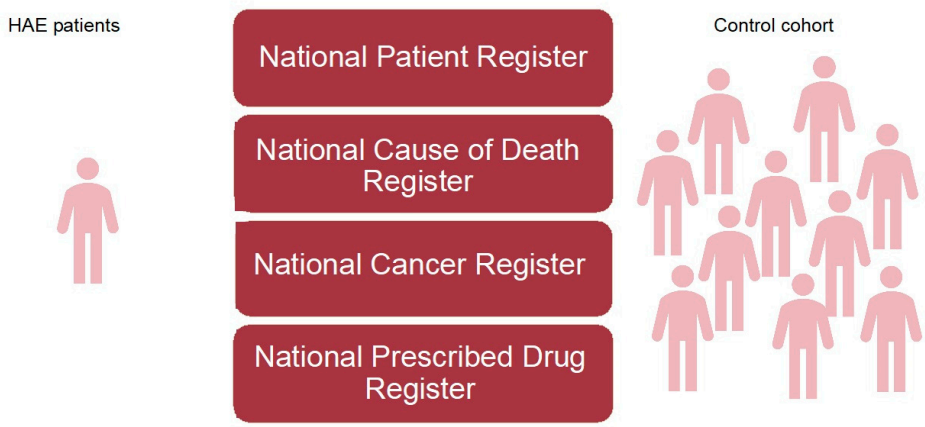

Figure 8. The HAE patient cohort and the control cohort with the ratio 1:10 were compared in the National Patient Register, the National Cause of Death Register, The National Cancer Register and the National Prescribed Drug Register.

The Swedish registers started at different times. The National Patient Register provides information on all completed inpatient care since 1964, registering diagnoses by International Classification of Diseases (ICD) codes. In 1987 the register became nationwide and since 2001 it also includes specialized outpatient care. The National Cause of Death Register contains data from 1961, whereas the National Cancer Register started in 1958. The National Prescribed Drug Register started as late as July 2005.

In the present study, diagnostic codes from International Classification of Diseases, the 9th revision (ICD-9; 1987-1996) and the 10th revision (ICD-10; 1997-present) were included, which means information from all the included registers from 1987 until the end of 2019 was used. There is no outpatient register in use, which may have an impact on the diseases usually treated in the outpatient care, such as hypertension, hyperlipidemia, asthma, allergy, and most infectious diseases, for example pneumonia and tonsillitis. This was one of the reasons why we did not include infections in the current study.

Of the 239 HAE patients in the cohort, 146 were already included in a cohort study that was conducted 2007-2016[27]. Of this initial cohort, 12 patients were deceased by 2019 when data was collected in this present study.

In paper III we aimed to investigate the extent as well as type of medical treatment in HAE patients, both disease-specific and possibly disease-modifying drugs, and how the treatment has evolved during the study period (2005 – 2019). The National Prescribed Drug Register (*Läkemedelsregistret*) started in July 2005 and covers registrations of prescription and collection of pharmaceutical drugs from both primary and secondary care. In this study, we obtained data regarding number of packages, dosage, volumes, expedition dates and collection for each individual and ATC code. The percentage of individuals collecting drugs each quarter was determined. We were also interested in the most recent numbers regarding age and gender distribution of the different drugs. This information was obtained by determining the collection of drugs during at least three quarters in 2019.

Paper IV

Paper IV was performed as a case-control study. Patient data were collected from 16 consecutive HAE patients treated at the Allergy Clinic, Skåne University Hospital in Lund. The patient cohort was compared with a control cohort consisting of 16 healthy volunteers matched for age and sex. Peripheral venous blood samples were collected from both HAE patients and controls. Complement analyses and flow cytometry were performed as for clinical routine samples at Clinical Immunology and Transfusion Medicine, Region Skåne, Lund. Concentrations and proportions of B cells, NK cells and T cells were determined by flow cytometry, as well as subphenotyping of B cells and T cells including proportions of different T

helper cell populations (Th1, Th2 and Th17). Analyses of whole blood with differential counts were performed as for routine clinical samples at Clinical Chemistry, Region Skåne, Lund. Commercial ELISAs were obtained from Quidel (C3a, iC3b and C5a), Svar Life Science (C4d) and Hycult Biotech (TCC). ELISAs were performed at Biomedical Centre (BMC) in Lund, using plasma samples frozen at -80 °C within 2 hours of sampling.

Ethics

Study I, II, III and IV were approved by the Swedish Ethical Review Authority (2019-01623) and (2020-05106) for study IV, and executed in accordance with the Helsinki declaration. In study IV written informed consent was obtained from all patients.

Statistics

In order to study time to first event for the specific outcomes of interest in the patient and control cohort in paper I and II, the Kaplan-Meier approach was used together with the log-rank test. By using Cox regression models, the corresponding hazard ratios between the cohorts were calculated with the assumption of non-proportional hazards. Data management was done in SAS version 9.4 (SAS institute, Cary, NC) and the statistical analysis was performed using R version 3.4.1 (R Foundation for Statistical Computing, Viennna, Austria). When studying associations between cases and controls, linear mixed regression models were used in order to account for the dependency over calendar year.

In paper III all plots were created using R 4.1.2 and ggplot2. Smoothed trend lines were fitted using LOESS.

In paper IV all statistical analyses were performed with GraphPad Prism 9.4.1 (GraphPad Software San Diego, CA) using Mann-Whitney U test for two-group comparisons, and Spearman´s correlation test for determining correlations. Data were calculated using medians and interquartile range. Mann-Whitney U test is a non-parametric method that is used to analyze a numerical variable without assuming a normal distribution, and particularly useful in small cohorts which was the case in this study (n=16). Spearman´s correlation test is also a non-parametric method, based on ranks, with the assumption that the numerical parameters may be skewed. Non-parametric methods are less efficient in detecting significant differences but more robust than parametric methods since they are less affected by extreme values. Results were considered statistically significant at $P < 0.05$.

Results and discussion

Paper I and II

Table 2. Demographic characteristics of the study population. Reprinted from paper I, table 1.

	HAE PATIENTS n = 239 (100%)	**REFERENCE POPULATION[a] n = 2383 (100%)[b]**
Male/female (%)	111/128 (46% / 54%)	1103/1280 (46% / 54%)
Age (years)[c]		
0-19	49 (20.5%)	490 (20.6%)
20-39	67 (28.0%)	663 (27.8%)
40-59	66 (27.6%)	660 (27.7%)
≥60	57 (23.9%)	570 (23.9%)
HAE type 1	216 (90.4%)	
Male/female	102/114 (47%/53%)	
Age (mean; SD)	43; 23 years	
HAE type 2	23 (9.6%)	
Male/female	9/14 (39%/61%)	
Agen (mean; SD)	39; 21 years	

Abbreviation: HAE, hereditary angioedema.
[a] Control subjects were matched for year of birth, sex and county of residence.
[b] For one patient, only three control individuals could be identified.
[c] At date of enrollment in the study (September 2020)

In this retrospective register-based study, we aimed to investigate comorbidities in HAE. Since C1INH is a main regulatory protein of several biological pathways including the complement system, the contact activation system, the coagulation system and the fibrinolytic system, chronic activation of these systems may result in comorbid conditions. Previous studies have presented a link between HAE and autoimmune diseases, in particular SLE, likely explained by consumption of C4[104]. There are also reports of thyroiditis[153-155,161], Crohn´s disease[162], rheumatoid arthritis[163] and glomerulonephritis[164,165] being associated with HAE. An increased level of autoantibodies in HAE patients have also been reported[166,167]. Regarding coagulation, several previous studies present increased levels of coagulation markers, in particular D-dimer[175-177]. However, no increased risk of thromboembolic events in HAE patients have been reported.

The results from the register-based studies demonstrated that HAE patients had an overall increased risk of autoimmune disease (AD) (OR 1.65; 95% CI 1.15-2.35), in particular SLE (OR 71.87; 95% CI 8.80-586.7) and autoimmune endocrine

diseases (OR 1.98; 95% CI 1.18-3.35). This finding was further supported by a significantly higher prescription of thyroid hormone substitution ($P < 0.001$). In a subgroup analysis, the cumulative incidence of ADs in males and females respectively, showed a significance in females only (P=0.02). A prevalence of 17.6% regarding ADs in general was found in the patient cohort compared to 11.5% in the control cohort. One previous study including 157 patients reported a prevalence of 12% of ADs in HAE patients[153]. Hence, previous reports on enhanced risk of autoimmune diseases were confirmed. It was also shown in the study that patients with HAE had an increased tendency of having two or more ADs (P=0.017).

A two-fold higher prevalence of allergy and asthma was seen for the first time in HAE patients compared to controls. There is a possibility that selection bias may contribute to the increased prevalence of these diseases since many HAE patients are treated at allergy clinics. These diseases are usually treated in the primary care, whose diagnoses are not included in the Swedish National Patient Register, possibly resulting in skewed conclusions regarding such diagnoses. However, we noticed a tendency to a higher prescription rate of asthma and allergy medication, i.e. inhaled β2-agonists, inhaled corticosteroids, and oral antihistamines among HAE patients, although not reaching significance.

In long-standing inflammatory diseases like rheumatoid arthritis and SLE, there is an increased risk of cancer[185-187]. However, the risk of cancer was not enhanced in HAE patients in the present study.

An increased risk of cardiovascular disease in HAE patients was demonstrated, including arterial thromboembolism (ATE) (OR 6.74; 95% CI; 1.89-24.06), venous thromboembolism (VTE) (OR 4.20; 95% CI 2.42-7.23) and hypertension (OR 1.64; 95% CI 1.12-2.39), supported by a significantly higher prescription of antihypertensive medication (P<0.001). A higher risk of hyperlipidemia (OR 2.01; 95% CI 1.16-3.50) was also shown, which was supported by a higher prescription of lipid-lowering medication (P<0.028).

To further evaluate the thrombotic pathologies, we looked at composite VTE including ICD-codes for deep vein thrombosis (DVT), pulmonary embolism (PE), thrombophlebitis, portal vein thrombosis and other VTE, and composite ATE including ICD-codes for cerebral infarction, ischemic heart disease and other ATE in HAE patients compared to the controls. This analysis revealed that HAE patients are at increased risk of VTE but not ATE (Figure 9).

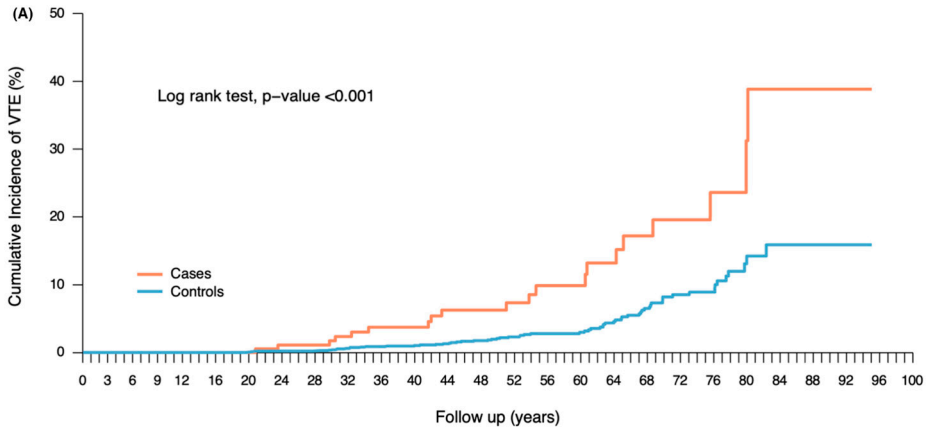

Figure 9. Cumulative incidence of composite venous thromboembolism (VTE) in cases with hereditary angioedema (HAE) vs. controls represented as a percentage of the total population. *P*-values for log rank tests are provided. Reprinted from paper IIa, figure 2.

This is the first study to show a connection between HAE and VTE, although previous studies have shown an increase in plasma levels of coagulation biomarkers such as D-dimer, prothrombin fragment 1 + 2 and thrombin-antithrombin complexes in HAE patients both during attacks and in remission[176].

After publishing these results, a comment from a research group was published[188] proposing that the conclusion of the results is likely confounded by indication and misclassification of VTE and therefore should be interpreted with great caution. The reasons mentioned were enhanced performance of imaging because of elevated D-dimer in these patients, enhancing the detection of incidental thrombotic finding, and that we could not adjust for the use of medication (tranexamic acid and i.v. administered C1INH concentrates). We replied that, after reanalyzing our data, excluding portal vein thrombosis and thrombophlebitis, in light of concerns over potentially misclassified atypical VTE events, the odds ratio remained significant (OR: 3.06, 95% CI: 1.68-5.56, $P < 0.001$), similar to the OR in the original analysis (OR: 3.59, 95% CI: 2.17-5.84, $P < 0.0001$). In the light of this, misclassification of VTE seems unlikely. Regarding the suggestion by Petersen *et al*. that tranexamic acid may contribute to VTE, we commented that there is no clear evidence of association with usage of tranexamic acid and VTE[189]. There is also no correlation between treatment with C1INH concentrates and VTE, in fact there is evidence of reduced incidence of VTE associated with treatment of C1INH, both in a clinical and preclinical setting[183,190]. We further speculated that since coagulation biomarkers are elevated not only during attacks, but also in remission in HAE patients[177], a systemic chronic activation of coagulation may be suggested in these patients, leading to a possible enhanced risk of VTE. This finding challenges the dogma that HAE is not associated with thrombotic pathologies.

Paper III

In paper III we aimed to investigate the trend in medical treatment of HAE patients in Sweden during the past decades. Around the time of the start of the National Prescribed Drug Register, which started in July 2005, a few new treatments became available which is a fruit of decades of intense research.

We specifically aimed to investigate long-term prophylaxis and on demand treatment for HAE patients, as well as other prescribed drugs that have the potential to interfere with disease control in HAE, such as angiotensin converting enzyme (ACE) inhibitors[191] and oral contraceptives[192]. In order to get as close to the truth as possible in a register-based study, we obtained data on the collection of the prescribed medicines. Medicine collection from pharmacies may serve as a surrogate measure of compliance to prescription.

Our findings showed that 50% of all Swedish HAE patients were on long-term prophylaxis (LTP). The LTP most commonly used was pd C1INH, used by approximately 30% with a steady increase. Since pd C1INH also is used as an on-demand treatment, we considered a dosing of ≥ 24,000 IU/quarter as prophylactic treatment. There was a predominance in prescription of C1INH to younger females. Tranexamic acid was used by approximately 10%, with a predominance to younger females and children. Androgens were collected by approximately 10% of the patients despite concerns about adverse effects[59] with a slight preference to males, and most frequently in the form of danazol (Figure 10).

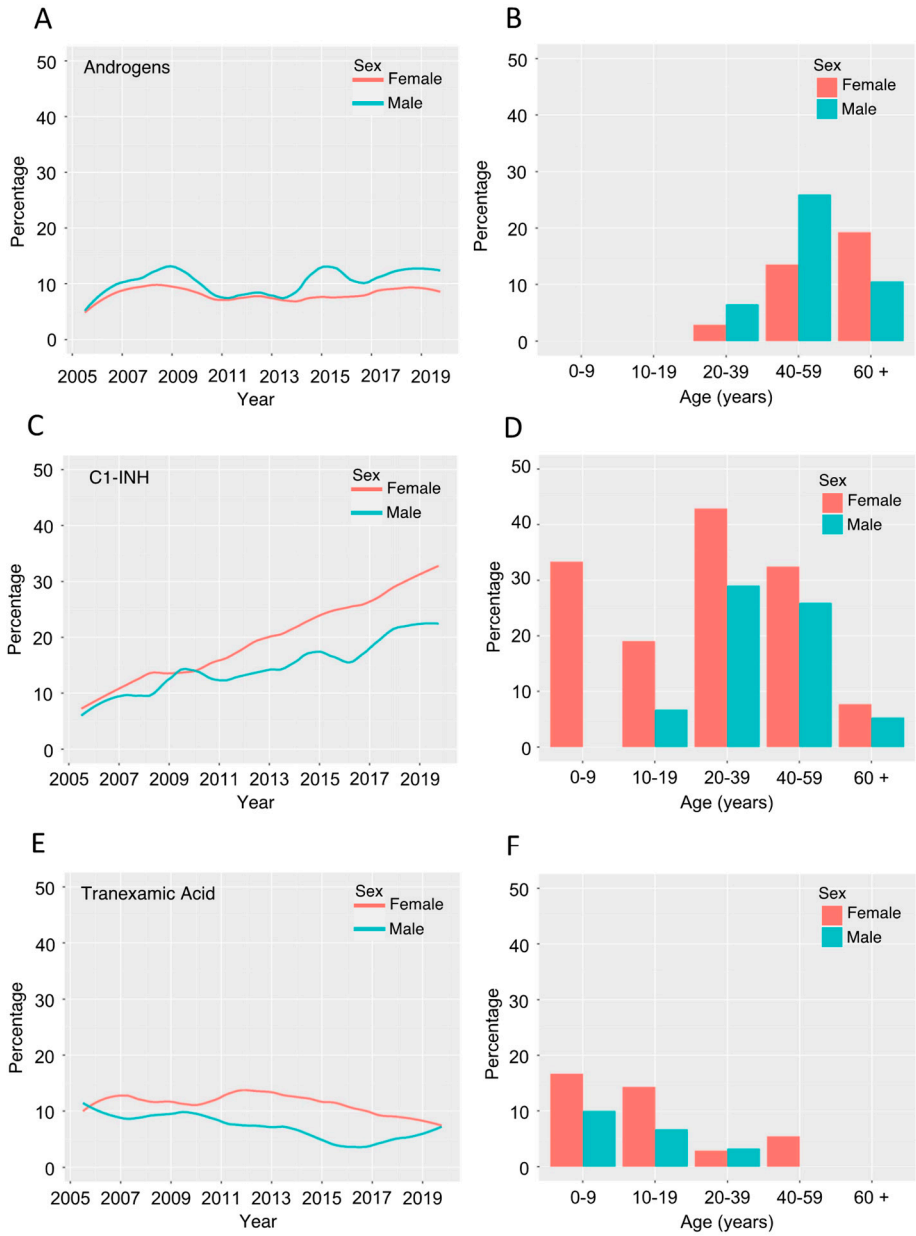

Figure 10. Long-term prophylaxis of HAE (2005-2019). A. Individuals who obtained prescriptions for attenuated androgens (danazol/oxandrolone) each quarter. B. Age/sex of individuals treated with attenuated androgens in 3 or more quarters in 2019. C. Prophylactic C1INH (≥24,000 IU/quarter). D. Age/sex of individuals treated with attenuated androgens in 3 or more quarters in 2019. E. Tranexamic acid. F. Age/sex of individuals who obtained prescriptions for tranexamic acid in 2019. Reprinted from paper III, figure 1.

Here, the assessment of on demand treatment was made based on the prescription of icatibant only, which was introduced in 2010 in Sweden. The definition of poorly controlled HAE was set to collection of ≥ 6 doses of icatibant per quarter. Since 2010, an increasing proportion of patients were prescribed icatibant with as much as 45% of the females and 35% of the males in 2019, indicating that women are affected by HAE angioedema episodes more frequently than men.

Since one of the findings in the comorbidity study (paper I) pointed at hypertension as being one of the more common comorbidities in HAE patients, we were interested in assessing the prescription of hypertension medicines. ACE inhibitors are known to evoke angioedema, usually affecting the face or tongue, in 0.1-0.7% of its users[193]. This phenomenon is called ACE inhibitor-induced angioedema. The mechanism of angioedema induced by ACE inhibitors is that ACE is involved in the degradation of bradykinin, and ACE inhibitors should therefore not be used in HAE. In this study, fortunately, there were only few reports on usage of ACE inhibitors.

Estrogen has a negative impact on angioedema by increasing kininogenase activities[194]. There is a general consensus not to use estrogen-containing oral contraceptives in HAE patients. In this study we found a decline of this prescription during the observation time, reaching zero in 2019. On the other hand, the collection of progestins increased. Progestins have shown a positive effect on reduction of angioedema[195].

A strength of the registry-based studies is that they are relatively large, both regarding the number of patients (239) considering that HAE is a rare disease, as well as the large number of matched controls per patient (10). Another strength is that validation studies of the National Patient Register indicate a high coverage, above 98%, and a high percentage of correct diagnoses[196]. A major limitation of these studies is that we did not have access to the patients´ medical records, lacking additional information about other diseases, medications, provoking events and cardiovascular risk factors such as smoking. As mentioned earlier, a drawback of the study is that diagnoses from primary care, where many diseases are treated, are not included in the National Patient Register which could result in selection bias of some of the comorbidity diagnoses for HAE patients who are treated at specialized outpatient care.

Paper IV

We aimed to investigate the lymphocyte distribution, complement and complement fragments among HAE patients by comparing 16 patients with HAE1 with 16 matched controls. We were also interested in any differences in lymphocyte populations between patients with high disease activity and low disease activity. High disease activity was defined as ≥ 2 angioedema attacks per month. One

important question was whether differences in the lymphocyte populations can provide information regarding the risk of acquiring comorbidities. Although only three of the 16 patients had an autoimmune disease, there might be a difference in lymphocyte subsets also in HAE patients without autoimmune disease, possibly leading to predisposition for autoimmune disease. Nine of the 16 patients had autoantibodies.

The results of this study showed that patients with HAE had decreased number and proportion of NK cells in peripheral blood compared to controls (Figures 11 and 12).

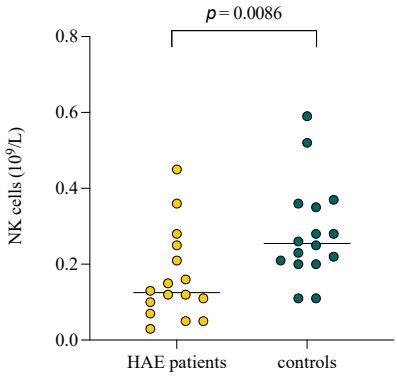

Figure 11. NK cell count in HAE patients and matched controls. Reprinted from paper IV, figure 2a.

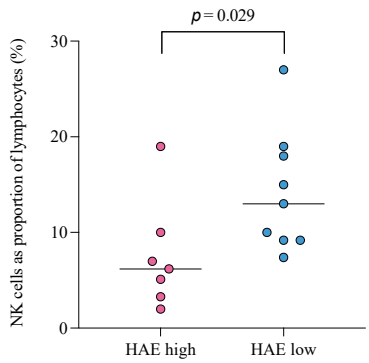

Figure 12. Proportion of NK cells in HAE patients with high and low disease activity. Reprinted from paper IV, figure 2c.

There was a positive correlation between the proportion of NK cells and the level of C1INH (Figure 13) and an inverse correlation to disease activity, as patients with a high disease activity displayed a lower percentage of NK cells.

As discussed previously, a low number of NK cells has been described in several studies in SLE patients as well as in rheumatoid arthritis, diabetes type 1 and psoriasis[170].

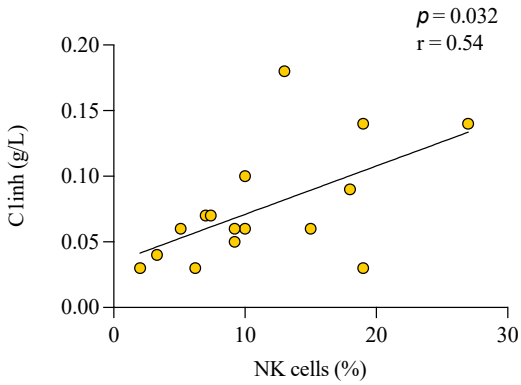

Figure 13. Positive correlation between level of C1inhibitor and percentage of NK cells in HAE patients. Reprinted from paper IV, figure 3a.

A difference was observed in the balance between Th1 and Th2 cells, with a predominance of Th2 cells in HAE patients compared to healthy controls (Figure 14). In paper I we found a significantly higher risk of allergy and asthma, diseases known to be associated with an emphasis of Th2 cells.

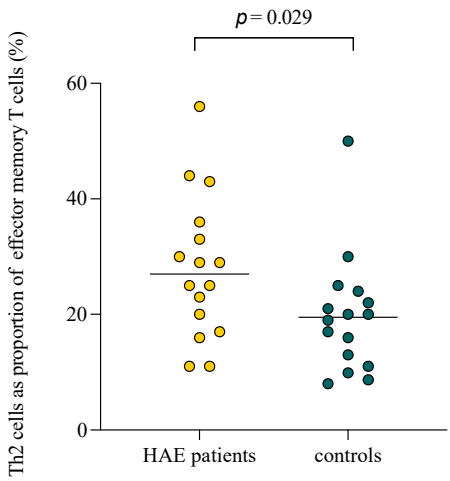

Figure 14. Proportion of Th2 of effector memory T cells in HAE patients and matched controls. Reprinted from paper IV, figure 4b.

A low NK cell count in combination with a Th2 cell predominance in HAE patients is interesting. Previous studies have demonstrated that NK cells, producing IFN-γ, might counterbalance and suppress type 2 inflammation. Low NK cell numbers may be an explanation to why HAE patients are prone to type 2 inflammatory diseases

and humoral immune response producing antibodies, as in allergy, asthma, and autoimmune diseases.

A limitation of this study is the small number of patients (16) included. A strength of the study is that the controls were matched by sex and age, which means results would not be influenced by age- and sex-related differences in lymphocyte distribution.

Conclusions and future aspects

Paper I and II

The register-based studies demonstrated an increased risk of a number of comorbid conditions in HAE patients, i.e. autoimmune diseases, allergy, asthma, VTE, hyperlipidemia and hypertension.

These results give rise to a number of questions. To what extent are comorbidities dependent on the disease itself, for example frequency or severity of attacks? Does the level of C1INH or C4 affect the risk of developing comorbidities? Do LTPs modify the risk of comorbidities?

To further investigate the extent and distribution of autoimmune diseases as well as autoantibodies and complement analyses in patients with HAE1 and 2, we have recently started a nation-wide study. To date, 31 patients have been included, of which 55 % have autoantibodies, and 16 % have ADs. The most common autoantibodies are against thyroid peroxidase (TPO), β2-glycoprotein I (GPI), rheumatoid factor (RF), and antinuclear antibodies (ANA). The high percentage of patients with autoantibodies is in line with previous studies[166,167]. Preliminary results of the national study also show that 50% of the HAE patients investigated so far have allergic symptoms verified by the presence of specific IgE.

An important aspect to consider is that autoantibodies are typically present many years before the onset of symptoms in SLE, with ANA being the first biomarker to appear[197]. This may indicate a need for regular screening for the presence of autoantibodies in HAE patients, at least with regard to diseases where the vulnerability is higher, e.g. age, sex, and heredity.

Regarding the increased risk of VTE, more studies are needed to confirm the association between HAE and VTE. Will the level of C1INH be a useful factor determining the choice of LTP, or are there other factors to consider, such as the presence of autoantibodies (e.g. antiphosphoplipid antibodies)? In the light of C1INH being a negative regulator of the intrinsic coagulation pathway, investigation of the correlation between VTE and the level of C1INH would be relevant to investigate in a wider context, not only in HAE patients.

Paper III

In this study approximately 50% of the Swedish HAE patients were shown to use LTP. Substitution with pd C1INH was the most common treatment of choice, used by 30% of patients. Secondly, attenuated androgens and tranexamic acid were used by approximately 10% of patients respectively. On demand treatment was prescribed both to a higher percentage of females (45%) than of males (35%), and also with a higher number of doses. This indicates that women are more frequently affected by angioedema attacks than men.

Shortly after this study was completed, long term prophylactic therapies targeting kallikrein were introduced in Sweden. In addition, a number of novel treatment options with new targets are on the way, such as FXIIa inhibitors. It will be highly interesting to perform follow-up studies in a few years, investigating whether new LTP modalities will improve health-related quality of life, the need for on demand medication and, not least, the spectrum and incidence of comorbidities.

Considering HAE treatment in relation to comorbidities, we are currently investigating diagnoses in relation to different LTPs during the time period 2005-2019.

Paper IV

This study demonstrated a decreased number of NK cells and a Th2 cell predominance in peripheral blood of HAE patients compared to controls. The degree of these differences correlated with disease activity. HAE patients with a higher disease activity had a higher proportion of transitional naïve B cells. HAE patients in general had a lower proportion of class-switched memory B cells as well as a lower number and proportion of plasmablasts compared to controls.

Transitional B cells seem to be altered in several autoimmune diseases, but in different ways, although in SLE they seem to be increased. Transitional B cells are reported to prevent the differentiation of $CD4^+$ T cells into Th1 and Th17 cells[198].

More studies are needed in the area of immune cells and cytokines in HAE. It would also be of interest to do a follow-up study, exploring possible changes in lymphocyte subsets related to changes in disease activity and treatment.

Populärvetenskaplig sammanfattning

Hereditärt angioödem (HAE) är en ärftlig sjukdom som drabbar ca 1: 50 000. Sjukdomen beror på brist på, eller nedsatt funktion av C1 inhibitor (C1INH) och orsakas av en förändring i genen som kodar för C1INH.

Sjukdomen kännetecknas framför allt av svullnadsepisoder (angioödem) som oftast drabbar huden i ansikte, händer, fötter och magtarmkanalens slemhinnor. Även andningsvägarna kan drabbas, vilket kan vara livshotande. Attackfrekvens och svårighetsgrad varierar under livets gång. Angioödemen uppstår på grund av aktivering av kontaktaktiveringssystemet, vilket leder till ökad produktion av bradykinin, som i sin tur ökar kärlgenomsläppligheten vilket ger ökat vätskeutträde till vävnaden. Det finns ett antal faktorer som kan utlösa attacker, såsom fysiskt trauma och stress, men oftast vet man inte varför man får en attack. De senaste 20 åren har det hänt mycket avseende forskning och utveckling av läkemedelsbehandling för HAE, och det pågår fortsatt intensiv forskning kring detta. Numera finns det både bra akutbehandling och förebyggande behandling (långtidsprofylax).

Dessa sjukdomsyttringar är uppenbara, och också det som karaktäriserar sjukdomen. Men med tanke på att C1INH är involverat i flera biologiska system är det rimligt att ställa sig frågan om inte denna brist även kan ge upphov till andra symptom eller sjukdomar. Det finns rapporter om associationer mellan HAE och vissa autoimmuna sjukdomar, framför allt sjukdomen systemisk lupus erythematosus (SLE) som är en autoimmun sjukdom som kan drabba många olika organ. Dock har det inte gjorts några större epidemiologiska studier eftersom HAE är en ovanlig sjukdom.

Vi ville därför undersöka samsjukligheten hos patienter med HAE. Genom personlig kontakt med i stort sett alla läkare i Sverige som behandlar patienter med HAE samt läkare som arbetar på kliniskt immunologiska laboratorier vilka bedriver diagnostik av sjukdomen, har vi sammanställt en kohort bestående av 239 patienter som lider av HAE, varav 227 levande år 2019. Med hjälp av Statistiska Centralbyrån identifierades 10 kontrollpersoner per patient, matchade för ålder, kön och länstillhörighet. Vi jämförde dessa 2 kohorter med flera av Socialstyrelsens register; Patientregistret, Cancerregistret, Dödsorsaksregistret och Läkemedelsregistret. Patientregistret registrerar alla slutenvårdstillfällen och innehåller också uppgifter om behandling i den specialiserade öppenvården.

I det första projektet ville vi undersöka samsjukligheten hos HAE patienter baserat på registrerade diagnoskoder enligt ICD-9 och ICD-10. ICD (International Statistical Classification of Diseases) är ett internationellt klassificeringssystem av sjukdomar. Vi fann en signifikant ökad risk för autoimmuna sjukdomar, i synnerhet SLE, hos HAE patienter jämfört med kontrollkohorten, vilket bekräftade tidigare fynd. Studien visade även att HAE patienter har en ökad risk för höga blodfetter, allergi och astma. Dessutom fann vi en ökad risk för hjärt-kärlsjukdomar, framför allt högt blodtryck och blodproppar. Studier har tidigare visat att HAE patienter har förhöjda markörer i blodet som tyder på aktivering av blodkoagulering.

När vi adderade alla diagnoskoder för venös tromboembolism (VTE) respektive arteriell tromboembolism (ATE) fann vi att risken att drabbas av VTE var omkring 3 gånger högre hos HAE patienter jämfört med bakgrundsbefolkningen, men det fanns inte någon ökad risk hos HAE patienter att drabbas av ATE. Detta publicerades, vilket föranledde ett svar på vår publikation från en forskargrupp som ifrågasatte resultatet baserat på huruvida diagnoskoderna var relevanta, eventuellt ökade fynd av tromboser på grund av fler röntgenundersökningar samt möjlig association till läkemedel som skulle kunna vara trombosfrämjande. Vi svarade på deras synpunkter, och kunde visa att skillnaden kvarstod trots att vi tog bort de diagnoskoder de ifrågasatte. En nackdel med studien är att vi inte har tillgång till patienternas enskilda journaler och vet därför inget om övriga riskfaktorer (till exempel rökning).

Behandlingen av HAE består av akutbehandling i form av icatibant som är en bradykinin 2 receptor antagonist som ges subkutant. Man kan också behandla akuta attacker med C1INH intravenöst. Det finns också förebyggande behandling i form av C1INH, tranexamsyra som ges i tablettform, och försvagat manligt könshormon (så kallade androgener) i tablettform. Efter arbetets slut har det tillkommit ytterligare förebyggande läkemedel med nya angreppspunkter. I arbete 3 undersökte vi hur behandling av HAE har sett ut sedan läkemedelsregistret startade 2005 fram till studiens slut år 2019. Resultaten visade att omkring 50% av HAE patienterna i Sverige behandlas med förebyggande mediciner; ca 30% behandlas med C1INH, 10% med tranexamsyra och 10% med försvagade androgener. Ca 40% av alla HAE patienter förskrivs med akutbehandling med icatibant. Det ska bli intressant att följa hur behandlingstrenden kommer att se ut framöver vad gäller både akut och förebyggande behandling, och genom nya studier se om de kan påverka samsjukligheten.

I det fjärde arbetet analyserade vi vita blodkroppar och flera faktorer i komplementsystemet hos 16 HAE patienter jämfört med 16 frivilliga kontrollpersoner, matchade för ålder och kön. Vita blodkroppar är en del av immunförsvaret, och samspelar för att på olika sätt bekämpa till exempel bakterier och virus. En del vita blodkroppar bildar antikroppar, och dessa kan också ge upphov till autoimmuna sjukdomar då immunförsvaret attackerar den egna vävnaden. Eftersom HAE patienter har en ökad risk för autoimmun sjukdom ville

vi se om man på cellnivå kan se skillnader i blodet hos HAE patienter jämfört med friska kontroller, och om det dessutom finns skillnader hos patienter med aktiv sjukdom jämfört med de som har en sjukdom i lugnt skede. Antalet NK celler var lägre hos HAE patienter jämfört med kontroller, vilket man även sett hos patienter som drabbas av vissa autoimmuna sjukdomar. Även andelen NK celler var lägre hos HAE patienter, och ännu lägre hos HAE patienter med aktiv sjukdom jämfört med de med lågaktiv sjukdom. Vi kunde även se en obalans i de grupper av vita blodkroppar som kallas Th1 och Th2 celler, där andelen Th2 celler var högre och andelen Th1 celler lägre hos HAE patienter jämfört med kontroller. Andelen Th2 celler är ofta förhöjd vid allergiska sjukdomar och astma, vilket vi såg en ökad risk för hos HAE patienter i det första arbetet.

Dessa fynd leder till nya frågeställningar. Kan sjukdomsaktiviteten ge upphov till skillnader på cellnivå, och kan det påverka risken för samsjuklighet? Är det möjligt att olika behandlingsformer av sjukdomen kan påverka samsjukligheten genom att modifiera sjukdomsaktiviteten, eller genom den substans som tillförs? Fler framtida studier behövs för att ge svar på dessa viktiga frågor.

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LINDA SUNDLER BJÖRKMAN
is a physician, specialized in Allergy and Clinical immunology at Skåne University Hospital in Lund, Sweden. She is a researcher at the Faculty of Medicine at Lund University. Her research field is in Hereditary angioedema, with a particular interest in comorbidities associated with HAE.

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