Journal of Autoimmunity 104 (2019) 102312

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Driving towards Precision Medicine for angioedema without wheals

Anastasios E. Germenis[a,*], Marco Cicardi[b]

[a] Department of Immunology & Histocompatibility, School of Medicine, University of Thessaly, Larissa, Greece
[b] Dipartimento di Scienze Biomediche e Cliniche Luigi Sacco, Universita Degli Studi di Milano, IRCCS ICS Maugeri Milano via Camaldoli, Italy

ARTICLE INFO

Keywords:
Alternative trials
Angioedema
Biomarkers
Global angioedema registry
mHealth apps
Precision medicine

ABSTRACT

Evidence accumulated over the last two decades indicates that recurrent angioedema without wheals constitutes a diverse family of disorders with a much higher complexity than was previously regarded. Indicatively, during the last two years, novel variants of three genes other than *SERPING1* and *F12* have been identified in association with hereditary angioedema. Most interestingly, functional studies of at least one of these variants (the variant c.807G > T of *ANGPT1* gene) imply the existence of a new disease endotype in which the altered bradykinin metabolism and function does not play a central role. Therefore, using conventional approaches, it seems that the complexity of this disease cannot be sufficiently elucidated and any attempt to interrelate its many diverse aspects seems unrealistic. Similar to other rare and chronic diseases, a Precision Medicine approach, discovering the right target and giving "the right drug, for the right patient, at the right time, every time" seems the optimal future practice. Herein, we review recent data challenging and dictating the need for a switch of angioedema research into high-throughput approaches and we present the expected advantages for better understanding of the disease and patients management.

1. Definition

The term angioedema without wheals refers to patients with a relatively rare, disabling and potentially life-threatening hereditary (HAE) or acquired (AAE) disorder presenting with recurrent attacks of subcutaneous or submucosal edemas which develop as a result of transient release of vasoactive peptides and increased permeability of blood vessels (Fig. 1) [1].

2. The growing complexity

Evidence accumulated over the last two decades indicates that, as a matter of fact, recurrent angioedema without wheals represents a diverse family of disorders with a much higher complexity than was previously appreciated. A well-defined etiopathogenesis and a precise diagnosis are at the moment available only for hereditary and acquired types of C1-inhibitor deficiency (C1-INH-HAE and C1-INH-AAE). The genetic deficiency is attributed to variants of C1-INH encoding *SERPING1* gene, and the acquired to autoreactive B cell clones that deplete C1-INH [2,3]. Less clarified is a clinically similar but not identical type of HAE described in 2000 [4,5] which is presenting with normal plasma levels of functional C1-INH and without *SERPING1* mutations (nlC1-INH-HAE). Around one third of these patients present *Factor 12* gene variants. During the last two years, variants in angiopoietin 1 (*ANGPT1*), plasminogen (*PLG*) and kininogen 1 (*KNG1*) genes have been found to segregate with angioedema symptoms in families with HAE and normal C1-INH [6–8]. The pathogenesis of angioedema associated with these variants needs to be elucidated. For the majority of patients with HAE and normal C1-INH, the etiology remains unknown (U-HAE). The same applies to two types of acquired angioedema that are defined as idiopathic: one is prevented by antihistamine (IH-AAE) therapy while the other is not (InH-AAE) [2].

In recent years, enormous research efforts have led to the discovery of new drugs for HAE treatment with very different way of action, ways of administration, and risk to benefit ratios [9–11]. At the moment, six drugs for the treatment of HAE are in the market (Table 1), a seventh one is on phase 3 study and additional ones are on early preclinical or clinical development. Probably, such an abundance of different therapeutic modalities does not exist for any other rare disease! Based on the definitively proved pathogenetic mechanism of C1-INH-HAE, two of these drugs (plasma-derived and recombinant C1-INH) are used as replacement therapies while the others have been developed to block the production or the activity of bradykinin which is the proven principal mediator of disease symptoms. Recent advances, however, indicate

* Corresponding author. Department of Immunology & Histocompatibility, School of Medicine, University of Thessaly, Panepistimiou 3, 41500 Biopolis, Larissa, Greece.
E-mail address: agermen@med.uth.gr (A.E. Germenis).

https://doi.org/10.1016/j.jaut.2019.102312
Received 17 July 2019; Accepted 24 July 2019
Available online 09 August 2019

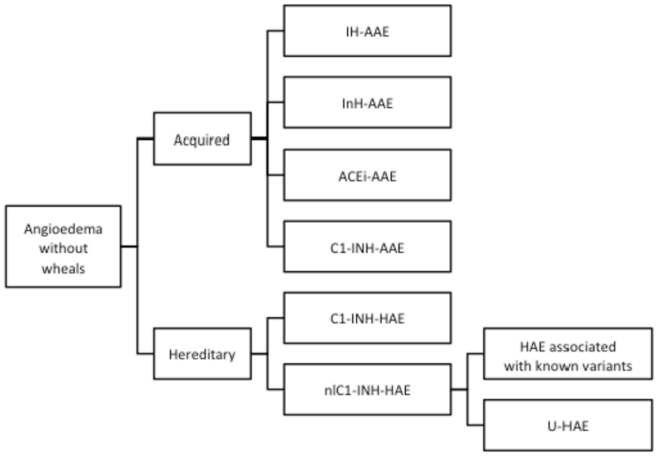

Fig. 1. Current classification of angioedema without wheals. (Adapted from Cicardi et al. [1]; for abbreviations see in the text).

that, beyond bradykinin, other mediators of endothelial function, like angiopoietin 1 [6], could be equally significant players in the HAE pathogenesis, signifying the existence of new or supplementary disease endotypes and potential targets for therapeutic intervention.

The same holds true in regard with some pathophysiological aspects of the disease that have been under-emphasized. For example, since 1986, autoantibodies against C1-INH has been recognized as a causative factor of acquired C1-INH deficiency and angioedema (type II AAE) [12]. However, their role in the development of angioedema associated with lymphoproliferative disorders (type I AAE) [13,14] as well as the effect of treatments directed against B cells (rituximab) in the control of autoimmune angioedema remain controversial [15,16]. Contrary, initial efforts towards uncovering a possible role of anti-C1-INH antibodies in C1-INH-HAE uncovered a strong positive correlation between the titers of the IgM type anti-C1-INH antibodies and the severity of the disease [17]. Taken together the above data indicate that a reconsideration of anti-C1-INH antibodies under the light of natural autoimmunity [18] would provide new insights on the pathophysiology of angioedema and probably new pathways towards its management. To this point it is worth mentioning that as early as 1985, Geha et al. [19] had implicated idiotype-antiidiotype interactions as a mechanism for the development of AAE in lymphoproliferative disorders.

Not surprisingly, therefore, trials for registration of new drugs did not result in definite therapeutic indications. The great proportion of HAE patients worldwide continue to be treated on a trial-and-error basis while adapting available treatments for the enormous clinical disease variability is far from being perfect. Thus, surveys have shown that many patients still report poor disease control followed by substantial morbidity, psychological stress, and decreased productivity and poor quality of life [20]. Moreover, this enrichment of the medical armamentarium is escalating healthcare costs limiting its availability to a restricted number of patients.

Ground gained by the Precision Medicine approach of other rare and chronic diseases is promising that the ultimate goal of discovering and giving “the right drug, for the right patient, at the right time, every time” [21] could be more effectively achieved by a switching of basic and clinical research of angioedema towards this new paradigm of medicine. In this perspective, we review recent data challenging and dictating the need for a switch of angioedema research into high-throughput approaches and we present the expected advantages of such an attempt for the better understanding of the disease and the more effective management of patients.

3. Paving the way to a new taxonomy

Till the turn of the century, variants of the *SERPING1* gene were the only disease-causing genetic defects that had been identified. Up to now, more than 500 different *SERPING1* defects associated with C1-INH-HAE have been reported which are leading to antigenic or functional C1-INH deficiency [22]. Such deficiency results in poor control of local activation of contact system resulting in enhanced generation of bradykinin at the site of the attack [23]. The increase in vascular permeability derives from the stimulation of bradykinin receptor 2 (B2R) which is constitutively expressed on endothelial cells (Fig. 2). Selective blockade of these receptors reverts attacks in C1-INH-HAE patients [24]. Bradykinin activates intracellular pathways that enlarge endothelial junctions with accumulation of extracellular fluid. *In vitro* experiments suggest that bradykinin receptor 1 (B1R), expressed upon inflammatory stimuli, and the gC1q receptor (gC1qR), which localizes contact system components on the endothelial cell surface, may also contribute to angioedema attacks [25]. Upon bradykinin stimulation, B1R and B2R transduce intracellular signals that activate endothelial nitric oxide synthase (eNOS) eventually leading to retraction of endothelial cell junctions, fluid extravasation and formation of local edema. Changes in number and type of receptors expressed on endothelial cell may be the key for understanding how specific triggers drive a constant genetic defect into a clinical phenotype characterized by periodic local symptoms [26].

On the other hand, the pathogenicity of all unexceptionally the *SERPING1* mutations that have been found associated with C1-INH-HAE, is debated. Proper functional or segregation studies cannot be implemented because of the large number of individual mutations and the absence of significant numbers of relatives. Another difficulty against the reliability of functional studies comes from the fact that some variants may have different consequences in different cell types while C1-INH structure-function correlations do not always interpret adequately the linkage between specific variants and the disease phenotypes. Thus, it remains elusive whether all *SERPING1* variants regarded so far pathogenic for HAE, are isolated or are expressed in parallel with either functional variations on genes involved in the metabolism or function of bradykinin, or represent *SERPING1* polymorphisms and mutations with as yet unknown functional consequences.

Accumulating data support the implied above complexity being many times even subversive of previously established concepts about C1-INH-HAE. For example, despite the fact that C1-INH-HAE is considered a dominant disorder, a case of C1-INH deficiency with recessive inheritance has been recently reported that, moreover, presents with C1-INH levels fluctuating from extremely low to completely normal values [27]. Another interesting issue of C1-INH-HAE genetics is that, in a small percentage of patients no *SERPING1* alterations can be found even after thorough analysis of the coding region [28,29]. Very recently, however, by the use of a next-generation sequencing (NGS) platform covering > 70% of the *SERPING1* intronic areas [30], we described a deep intronic mutation located in intron 1 (c.-22-155G > T, 155 nucleotides from the donor site) that was cosegregated with the disease in a C1-INH-HAE family. Bioinformatics analysis and preliminary functional data indicate that this is the disease-causing variant [31].

In the year 2006, two variants of the *F12* gene encoding blood-coagulation factor XII (FXII or Hageman factor), namely the Thr328Lys and the Thr328Arg missense mutations, were suggested as the causative defect in five families with nlC1-INH-HAE [32]. Latter studies revealed a few other mutations affecting the same proline-rich region of the FXII protein as the point mutations and co-segregating with the disease. These included a deletion of 72 bp (c.971_1018 + 24del72) [33] and a duplication of 18 bp (c.892_909dup) [34]. The above four *F12* variants account for about a third of nlC1-INH-HAE cases (classified as FXII-HAE). Contrary to C1-INH-HAE, the autosomal-dominant inheritance of FXII-HAE presents an incomplete penetrance [35]. FXII, a serine protease whose expression is increased by estrogen, is involved in the release of bradykinin as the activated enzyme has the capacity to cleave

Table 1
Therapeutic modalities for C1-INH-HAE.

Drug	Mode of action	Therapeutic indication	Route of administration
On the market			
Plasma-derived C1-INH	Replacement	ODT, STP, LTP	Intravenous
Recombinant C1-INH	Replacement	ODT, STP	Intravenous
Plasma-derived, volume-reduced C1-INH	Replacement	LTP	Subcutaneous
Icatibant (synthetic peptide)	Bradykinin B2 receptor antagonist	ODT	Subcutaneous
Ecallantide (recombinant protein)	Plasma kallikrein inhibitor	ODT	Subcutaneous
Lanadelumab (mAb)	Fully human mAb targeting plasma kallikrein	LTP	Subcutaneous
On phase III study for LTP/on phase II for ODT			
BCX7353	Plasma kallikrein inhibitor	ODT, LTP	Oral

ODT: on-demand treatment; STP: short-term prophylaxis; LTP: long-term prophylaxis; mAb: monoclonal antibody.

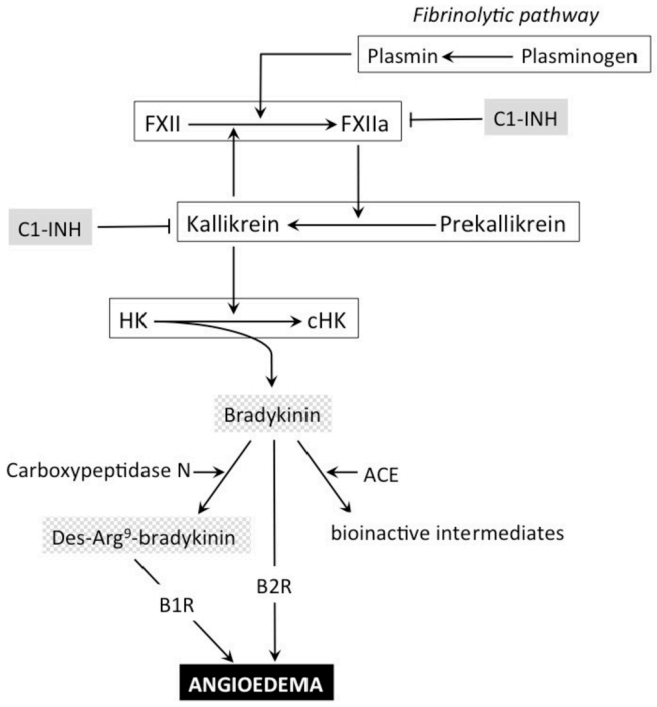

Fig. 2. Simplified diagram of the contact system.

plasma prekallikrein generating kallikrein, an enzyme responsible for the release of bradykinin from high molecular weight kininogen (Fig. 2). All FXII-HAE causing mutations introduce one or more cleavage sites into the FXII molecule that strongly amplify the rate of mutant FXII activation by plasmin, rendering thus C1-INH ineffective at preventing excessive bradykinin production [36,37]. Moreover, the two point mutations and the deletion were also reported to introduce new sites in the protein that are sensitive to enzymatic cleavage and therefore activation by plasmin, rendering C1-INH inefficient at hindering bradykinin overproduction [38]. Recently, Ivanov et al. [39] showed that FXII with Lys/Arg substitutions for Thr309 can be cleaved by thrombin and factor XIa to generate the truncated species δFXII activation of which by kallikrein is markedly enhanced when compared with factor XII, increasing kininogen cleavage *in vivo*. Despite the fact that FXII-HAE attacks are indistinguishable from C1-INH-HAE attacks, FXII-HAE is different from C1-INH-HAE as well as from nlC1-INH-HAE but without *F12* mutations (classified as unknown HAE, U-HAE) in many aspects, like the age at disease onset, the female gender predominance, the triggering effect of estrogens, the frequency of attacks, etc. [40,41].

Last year, Bafunno et al. [6], after performing whole-exome sequencing in a large multiplex family with U-HAE, presented evidence convincing that a missense mutation (c.807G > T) in the *ANGPT1* gene encoding for angiopoietin-1 is the causative defect for a new type of HAE. This finding implies the existence of a new HAE endotype in which the altered bradykinin metabolism and function do not play a central role. The authors suggest that the mutant ANGPT1 exposes reduced binding ability to the membrane receptor of the wild protein (tunica interna endothelial cell kinase-2, TIE2) and, thus, it cannot exert its normal function that is to stabilize the blood vascular endothelium. The resulting impaired regulation of endothelial barrier permeability drives the formation of swelling.

At about the same time, a previously non reported missense mutation (K330E) in exon 9 of the *PLG* gene, encoding plasminogen, was identified in nlC1-INH-HAE patients without *F12* mutations by the use of NGS [42]. Using similar genomic approaches, Bork et al. [7] carried out a family segregation study providing evidence that this *PLG* mutation is associated with a new type of angioedema transmitted as an autosomal dominant trait and featured by a high risk of tongue swellings. Functional data presented thereafter by Dewald [43] provided strong support about the pathogenicity of this variant. Contrary to what happens with ANGPT1-associated HAE, the frequency of this type of HAE seems to be up to 5% among U-HAE cases [44–47].

Very recently, one more potentially disease-causing variant was detected among U-HAE patients [8]. It is about the hitherto unknown variant c.1136T > A in exon 10 of the *KNG1* gene that was found cosegregated with the disease in a large U-HAE family and transmitted in a dominant inheritance pattern. The variant was not present in any of the 135 index patients of nlC1-INH-HAE families we analyzed (unpublished results).

The last publications mark the beginning of a new era in angioedema research grounded on the promise of genomic technologies (and probably of other omics) for a new HAE taxonomy that is a main component of Precision Medicine in clinical practice. As indicated by the first of the above cases, this new taxonomy will be based on underlying molecular mechanisms that may involve many other “players” of vascular permeability, beyond contact system, and will be able to identify and to represent the molecular etiology of HAE at a subtype or, even more, at an individual level. Obviously, some of the HAE subtypes to be discovered will continue reiterating established clinical diagnoses (such as C1-INH-HAE). However, it is anticipated that the majority of these descriptively clustered HAE subtypes will constitute true innovative distinct entities, with associated potential clinical utility.

4. Biomarkers: are we there yet?

4.1. Biochemical biomarkers

In everyday practice, clinicians dealing with HAE patients are confronted with a series of problems, solving the vast majority of them on the basis of patients’ history and clinical examination or using exclusion or non-validated therapeutic criteria. Very few laboratory tests, namely the antigenic and functional C1-INH, C4, C1q and the anti-C1-INH antibodies, expose an acknowledged decision-making value derived from either their long-term empirical use or very preliminary

Table 2
Currently in use and potential biochemical biomarkers for angioedema.

Biomarkers	Pros	Cons
Currently in use diagnostic biomarkers		
C1-INH antigenic levels	Easy measurement; critical for the diagnosis of type I C1-INH-HAE and C1-INH-AAE	Can be influenced by replacement treatment; do not reflect clinical course or prognosis
C1-INH functional levels	Critical for the diagnosis of type II C1-INH-HAE	Different types of tests may yield different results; do not reflect clinical course or prognosis
C4 levels	Easy measurement; important for the diagnosis of C1-INH-HAE and C1-INH-AAE	Great variability in healthy individuals; do not reflect clinical course or prognosis
C1q levels	Easy measurement; important for the diagnosis of C1-INH-AAE	Transient decline may occur exceptionally in C1-INH-HAE
Anti-C1-INH antibodies	Important for the diagnosis of C1-INH-AAE	Inconsistent findings between laboratories
Potential biomarkers		
Plasma kallikrein activity	Discriminates histamine- from bradykinin-mediated angioedema	Assays need to be validated
Cleaved high molecular weight kininogen	Stable biomarker; discriminates C1-INH-HAE patients in resting conditions and during attacks; separate the patients with different frequency of angioedema recurrences	Laborious measurement
Protease-inhibitor complexes	Easy measurement; biomarker of contact system activation	Complexes are short-lived *in vivo*; artificially low in C1-INH deficiency
Cleaved zymogen proteases	Biomarker of contact system activation	Difficult measurement
Fibrinolytic markers	Easy measurement	Nonspecific; great variability

research evidence (Table 2).

When the evidence that bradykinin was the mediator of angioedema in C1-INH-HAE became relevant [48], the plasma levels of this peptide were considered as potential biomarker. In 1998, Nussberger et al. [49] showed that increased plasma levels of bradykinin mark attacks in patients with angioedema due to C1-INH deficiency. Nevertheless, the rapid generation of this peptide upon contact system activation and its very short plasma half-life (less than 30 s) make bradykinin plasma levels extremely sensitive to pre-analytical procedures questioning its clinical utility [50]. Problems in quantifying the involvement of the kinin releasing system are in part unraveled through measurements of the bradykinin-generating enzyme, the plasma kallikrein (pKK). This parameter is not as much sensitive as bradykinin to *in vitro* activation of the contact system and can be measured by different approaches. Sampling procedures in anti-protease cocktails minimize the problem and render the assays sensitive enough to account for the *ex vivo* levels of pKK activity. Quantification by western blotting of cleaved high molecular weight kininogen (cHK), one of the physiologic end-products of pKK enzymatic activity, discriminates C1-INH-HAE patients in resting conditions and during attacks [51]. The same parameter can also separate the patients with different frequency of angioedema recurrences [52]. Using an ELISA based method, Hofman et al. [53] showed that cleaved kininogen is a biomarker for bradykinin release in HAE. As an alternative to the *ex vivo* quantification of cHK, blood collection in absence of protease inhibitors can discriminate the capacity of plasma to protect from pKK activity. Using this approach, Banerji et al. [54] showed that therapeutic doses of lanadelumab, the anti-kallikrein monoclonal antibody approved for prevention of angioedema in C1-INH-HAE patients, prevent cleavage of HK in plasma. Lara-Marquez et al. [55] developed an assay that measures the capacity of plasma to generate pKK. With this assay they found that patients with different forms HAE generate significantly more kallikrein than normal subjects or patients with histaminergic angioedema.

Thus, tackling pKK provides data that correlate with disease state, but the parameters considered, so far, need to be refined in order to increase sensitivity and specificity. Recently, Deroux et al. [56], Farkas et al. [57], and Christiansen and Zuraw [58] reviewed the promising but very preliminary results of many investigators who have tested dozens of biochemical parameters in an attempt to discover clinically useful biomarkers. Great efforts towards discovering more specific biochemical biomarkers are based on the continuously accumulating knowledge of the contact system biology, following the so-called "candidate approach" for biomarker discovery [59]. In fact, a striking discrepancy exists between the effort directed toward the discovery of biochemical biomarkers for angioedema and the number of them with a promising clinical utility.

Considering the required versatility of biomarkers, a not negligible obstacle in the development of biomarkers related to the activation of contact system is the heat sensitivity of the various enzymatic analytes and the degree to which it affects their stability and the analytical reliability of their measurements.

4.2. Genetic biomarkers

As far as genetic biomarkers are regarded, recent association studies have shown that the concomitant carriage of mutations of other genes encoding for proteins involved in bradykinin generation and function are correlated with parameters related to the clinical expression of C1-INH-HAE. The functional promoter polymorphism *F12*-46C/T that results in decreased translation efficiency and to low plasma levels of FXII activity and antigen, as well as the functional polymorphism *KLKB1*-428 G/A that causes a reduced plasma kallikrein complex formation with high-molecular-weight kininogen, have been shown associated with a significant delay of the disease onset and a significantly lower probability of the need for long-term prophylaxis (LTP) [60,61]. More interestingly, evidence provided recently by Rijavec et al clearly indicates that the *F12*-46C/T variant influence the penetrance of C1-INH-HAE. The authors have shown that, among C1-INH deficient individuals, carriers of the CC genotype compared to those of the TT genotype have a 25-fold greater risk of developing the disease [81].

Not irrelevant could be the associations found between gene variants and the angiotensin-converting enzyme inhibitor-associated angioedema (ACEi-AAE) the underlying mechanism of which seems to be the blockage of bradykinin and substance P degradation by ACE, and possibly the impaired metabolism of bradykinin and des-Arg^9-bradykinin by aminopeptidase P [62] (Fig. 2). A candidate gene study by Pare et al. [63] of the largest published cohort of ACEi-AAE and ACE inhibitor-exposed controls, detected a strong association between increased risk of ACEi-AAE and a polymorphism in the neprilysin gene (*MME*). Neprilysin is a membrane metallopeptidase colocalized with ACE and metabolizing many vasodilator and vasoconstrictor peptides, including bradykinin [64]. A new drug inhibiting neprilysin recently reached the market for treatment of cardiac failure [65]. In agreement with the possibility that reducing activity of this enzyme could facilitate angioedema, patients taking this medication seem to increase susceptibility to angioedema as patients on ACEi.

These findings indicate that high-throughput approaches, like genome-wide association studies, taking advantage of newer

techniques, like parallel sequencing, array expression profiling, whole exome sequencing, RNA sequencing, parallel omics, etc., despite very cost-intensive, are anticipated to pick up valid biomarkers. The potential of these approaches would become even higher when they combined with bioinformatics for *in silico* candidate approaches or other advanced methodologies, like expression quantitative trait loci (eQTL) analysis. Moreover, taking into account the complex interactions among key elements of the contact system as well as the involvement of other biological systems in the pathogenesis of HAE (e.g. vasculature) modular transcriptome repertoire analysis (modular genomics) could be a greatly promising innovative approach towards uncovering angioedema biomarkers and disease endotypes.

In conclusion, the long way to the discovery of validated biomarkers for HAE remains as yet unpaved. The so many parameters up to now tested in observation studies or indicated by basic research must be evaluated in regard to their analytic and clinical validity, clinical utility, feasibility, and time- and cost-effectiveness using appropriately designed trials (see below) [66]. This will allow diagnostic, predictive, prognostic and therapeutic biomarkers to be discovered in favor of a more effective management of HAE patients and solid evidence to be provided from clinical trials evaluating the therapeutic value of existing and emerging drugs.

5. Feasibility of large-scale studies and patients' participation

The implementation of large-scale studies in HAE faces a series of obstacles due to the problematic following-up of patients and the required collection of longitudinal clinical data. Thus, despite the efforts made [67,68], standardized, validated and widely accepted measures of disease severity are still missing. The problem has been recently faced creating the HAE Global registry (HGR) (https://haeregistry.org/; ClinicalTrials.gov NCT03828279). HGR gathers accurate data from throughout the whole HAE community with data provided by patients and treating physicians. The type and the quality of data collection allows real time evaluation of disease severity and prospective determination of the natural history of HAE and how it can be modified by intervening treatments. HGR was established in April 2018, at present collects 1163 patients from 23 centers in 5 European countries, with 60% of the patients registering their attacks through an electronic application. These numbers are rapidly growing and by the end of 2019, we expect 11 countries to be present with global representation (Fig. 3).

The global registry could help answering another question spreading across literature related to the possible effect of environmental factors on the clinical expression of HAE. Geographical differences in HAE clinical manifestations as well as an association between ethnicity and the kind of *SERPING1* mutations, has been observed [29]. To this point, extremely interesting is a recent report by Caccia et al. [27] of a C1-INH mutant whose stability and functional activity appear to be finely tuned by environmental conditions (i.e. temperature, pH, oxidative stress), which could vary in situations of mild stress, such as hyperthermia or metabolic acidosis. Considered together with the variability in disease course through patients' life these findings indicate that environmental factors (exposome) may exert a significant effect on the clinical expression of the disease (phenome). Furthermore, modification of patients' lifestyle could alter the eliciting disease-exacerbating triggers (e.g. stress, minor trauma, etc.).

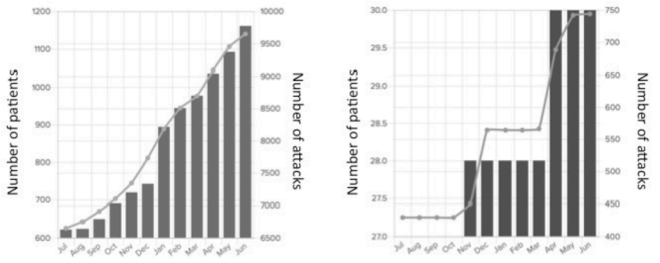

Fig. 3. Cummulative number of patients (columns) and attacks (line) recorded in the HAE Global Registry from all countries (left) and from Greece (right) since its opening.

The development of inventive strategies to collect and incorporate disease-relevant information by the use of advanced computational techniques could offer reliable answers to these questions. The upcoming practice of self-monitoring, known as the quantified-self movement, aims to empower patients to record daily activities and health-related information and, thus, holds much promise towards this direction [69]. Using mHealth apps, personal health devices and sensors/IoT (Internet of Things) artefacts, appropriate data capturing tools for patients and their caregivers can be provided. The technical artefacts employed for data capturing will be selected by engaging patients in the entire design and development process, e.g. through a user-centered design approach [70], thus achieving to fill a key objective of Precision Medicine, that is the participatory component [71]. Beyond disease-specific aspects, such as triggering factors, prodromal symptoms, frequency and severity of attacks, etc., such artefacts could include behavioral and social aspects, as well as more generic issues related with technology use, such as digital literacy and cultural aspects, as well as privacy, security and legal norms.

Empowering HAE patients to collect data at the phenome and the exposome level on a daily basis will provide further insights on treatment response, disease evolution, quality of life and disease burden, environmental conditions as well as behavioral aspects, all required and enabling the construction of robust Electronic Health Records [72].

Analyzing large number of data from single subjects has specific interest in rare diseases as it reduces the need for large series of patients. Main limitation to this approach is appropriate patient recruitment and continuous data collection. HAE is a clearly identified condition where presence of symptoms and disability alternate with absence. More than one hundred years after the description of the disease and in presence of several different drugs that revert and/or prevent symptoms, we still cannot answer the basic question on the average length of symptomatic periods. Simple information on attacks, prospectively collected by the majority of patients, could give the answer. The HGR already started collecting retrospective and prospective data that are expected to start appearing to help understanding the natural history of angioedema patients and the impact of the treatments [73].

6. Taking advantages from alternative trials

In recent years, clinical trials for emerging drugs for rare disease have been the subject of intensive discussions. Patient recruitment required for the implementation of robust randomized controlled trials (RCTs), the gold standard of generating strong evidence of clinical efficacy, is very difficult if not infeasible. Multicenter RCTs, on the other hand, is facing the possible heterogeneity of patient population due to inconsistent genotype-phenotype correlations and gene-environment interactions. To overcome these problems many alternative designs for clinical trials, like N-of-1 or single-subject trials, and methods for analysis have been proposed [74]. These are trials with multiple crossover (repeated measurements) and a repeat challenge-withdrawal design in which individual patients compare two or more treatments to one another [75] (Fig. 4). In the Oxford Centre for Evidence-Based Medicine levels of evidence [76], randomized and blinded N-of-1 trials have been listed as *level 1* evidence for individual treatment decisions.

The selection of primary and/or surrogate endpoints is a barrier that the implementation of N-of-1 trials usually meets in rare diseases. This is also true in the case of HAE where appropriately validated biomarkers are not available. There is no doubt that the frequency of attacks is indeed a well-defined, reliable and readily measurable primary endpoint but only for trials of drugs intended for LTP [77]. What, however, with trials performed to appraise the efficacy of drugs in the

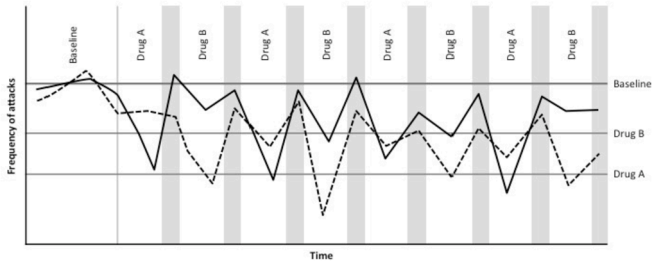

Fig. 4. Hypothetical outcomes associated with two individual n-of-1 trials investigating the efficacy of two different drugs, A and B, for HAE. The continuous and dashed lines represent the frequency of attacks for individuals 1 and 2, respectively, during the trial. According to the design, a baseline period was followed by four alternating periods in which two drugs were administered with a washout period (grey) between drug administrations. Note that individual 1 had a lower frequency of attacks while on drug A, as indicated by the horizontal lines denoting 'drug A' and 'drug B', representing the average frequency of attacks achieved while on the drugs. Individual 2 had a lower frequency of attacks on drug B. Adapted from Lillie et al. [80].

treatment of acute attacks where the usually used patient-reported symptom severity is a rather subjective measure [78,79]?

Till however validated biomarkers become available, the adoption of the N-of-1 trials logic in the everyday clinical care of selected HAE patients could benefit both patients and researchers. Such a transform could probe the myriad unclarified factors that shape not only the individual patients' response to particular treatments complexity but, even more, the enormous clinical variability of the disease. The knowledge of the complex landscapes of several angioedema types will be seriously expanded if multi-cycle within-patient crossover comparisons of drugs would be coupled with detailed biochemical measurements and high-throughput technologies of molecular profiling, such as mass spectrometry, gene-expression profiling and next-generation sequencing.

7. Conclusion

In conclusion, the above-mentioned aspects of HAE complexity cannot be sufficiently elucidated and any attempt to interrelate them seems utterly deadlock using conventional approaches, especially if a personalized medical care is anticipated. Once Precision Medicine seems tailored to the study of rare diseases, then HAE could be considered as a prototype for its implementation.

References

[1] M. Cicardi, W. Aberer, A. Banerji, M. Bas, J.A. Bernstein, K. Bork, T. Caballero, H. Farkas, A. Grumach, A.P. Kaplan, M.A. Riedl, M. Triggiani, A. Zanichelli, B. Zuraw, HAWK under the patronage of EAACI, Classification, diagnosis, and approach to treatment for angioedema: consensus report from the Hereditary Angioedema International Working Group, Allergy 69 (2014) 602–616 https://doi.org/10.1111/all.12380.

[2] M.A. Wu, F. Perego, A. Zanichelli, M. Cicardi, Angioedema phenotypes: disease expression and classification, Clin. Rev. Allergy Immunol. 51 (2016) 162–169 https://doi.org/10.1007/s12016-016-8541-z.

[3] M. Cicardi, A. Zanichelli, Diagnosing angioedema, Immunol. Allergy Clin. N. Am. 33 (2013) 449–456 https://doi.org/10.1016/j.iac.2013.07.001.

[4] K. Bork, S. Barnstedt, P. Koch, H. Traupe, Hereditary angioedema with normal C1-inhibitor activity in women, Lancet 356 (2000) 213–217 https://doi.org/10.1016/S0140-6736(00)02483-1.

[5] K. Binkley, A.E. III Davis, Clinical, biochemical, and genetic characterization of a novel estrogen-dependent inherited form of angioedema, J. Allergy Clin. Immunol. 106 (2000) 546–550 https://doi.org/10.1067/mai.2000.108106.

[6] V. Bafunno, D. Firinu, M. D'Apolito, G. Cordisco, S. Loffredo, A. Leccese A, M. Bova, M.P. Barca, R. Santacroce, M. Cicardi, S. Del Giacco, M. Margaglione, Mutation of angiopoietin-1 gene associates with a new type of hereditary angioedema, J. Allergy Clin. Immunol. 141 (2018) 1009–1017 https://doi.org/10.1016/j.jaci.2017.05.020.

[7] K. Bork, K. Wulff, L. Steinmüller-Magin, I. Braenne, P. Staubach-Renz, G. Witzke, J. Hardt, Hereditary angioedema with a mutation in the plasminogen gene, Allergy 73 (2018) 442–450 https://doi.org/10.1111/all.13270.

[8] K. Bork, K. Wulff, H. Rossmann, L. Steinmüller-Magin, I. Braenne, G. Witzke, J. Hardt, Hereditary angioedema cosegregating with a novel kininogen1 gene mutation changing the N-terminal cleavage site of bradykinin, Allergy (2019), https://doi.org/10.1111/all.13869.

[9] K. Bork, A decade of change: recent developments in pharmacotherapy of hereditary angioedema (HAE), Clin. Rev. Allergy Immunol. 51 (2016) 183–192 https://doi.org/10.1007/s12016-016-8544-9.

[10] H. Farkas, Hereditary angioedema: examining the landscape of therapies and preclinical therapeutic targets, Expert Opin. Ther. Targets 23 (2019) 457–459 https://doi.org/10.1080/14728222.2019.1608949.

[11] F. Perego, M.A. Wu, A. Valerieva, S. Caccia, C. Suffritti, A. Zanichelli, L. Bergamaschini, M. Cicardi, Current and emerging biologics for the treatment of hereditary angioedema, Expert Opin. Biol. Ther. 19 (2019) 517–526 https://doi.org/10.1080/14712598.2019.1595581.

[12] J. Jackson, R.B. Sim, A. Whelan, C. Feighery, An IgG autoantibody which inactivates C1-inhibitor, Nature 323 (1986) 722–724 https://doi.org/10.1038/323722a0.

[13] M. Cugno, R. Castelli, M. Cicardi, Angioedema due to acquired C1-inhibitor deficiency: a bridging condition between autoimmunity and lymphoproliferation, Autoimmun. Rev. 8 (2008) 156–159 https://doi.org/10.1016/j.autrev.2008.05.003.

[14] P. Triggianese, M.S. Chimenti, E. Toubi, E. Ballanti, M.D. Guarino, C. Perricone, R. Perricone, The autoimmune side of hereditary angioedema: insights on the pathogenesis, Autoimmun. Rev. 14 (2015) 665–669 https://doi.org/10.1016/j.autrev.2015.03.006.

[15] P.D. Ziakas, S. Giannouli, E. Psimenou, K. Evangelia, A.G. Tzioufas, M. Voulgarelis, Acquired angioedema: a new target for rituximab? Haematologica 89 (2004) e104–e105.

[16] M. Levi, C.E. Hack, M.H. van Oers, Rituximab-induced elimination of acquired angioedema due to C1-inhibitor deficiency, Am. J. Med. 119 (2006) e3–e5 https://doi.org/10.1016/j.amjmed.2005.09.018.

[17] L. Varga, G. Széplaki, B. Visy, G. Füst, G. Harmat, K. Miklós, J. Németh, L. Cervenak, I. Karádi, H. Farkas, C1-inhibitor (C1-INH) autoantibodies in hereditary angioedema. Strong correlation with the severity of disease in C1-INH concentrate naïve patients, Mol. Immunol. 44 (2007) 1454–1460 https://doi.org/10.1016/j.molimm.2006.04.020.

[18] S. Avrameas, H. Alexopoulos, H.M. Moutsopoulos, Natural autoantibodies: an undersugn hero of the immune system and autoimmune disorders - a point of view, Front. Immunol. 9 (2018) 1320 https://doi.org/10.3389/fimmu.2018.01320.

[19] R.S. Geha, I. Quinti, K.F. Austen, M. Cicardi, A. Sheffer, F.S. Rosen, Acquired C1-inhibitor deficiency associated with antiidiotypic antibody to monoclonal immunoglobulins, N. Engl. J. Med. 312 (1985) 534–540 https://doi.org/10.1056/NEJM198502283120902.

[20] A. Bygum, E. Aygören-Pürsün, K. Beusterien, E. Hautamaki, Z. Sisic, S. Wait, H.B. Boysen, T. Caballero, Burden of illness in hereditary angioedema: a conceptual model, Acta Derm. Venereol. 95 (2015) 706–710 https://doi.org/10.2340/00015555-2014.

[21] A. Ghannam, A.E. Germenis, C. Drouet, Hereditary kinin-mediated angioedema: current challenges, Aust. Intern. Med. 1 (2016) 1002.

[22] A.E. Germenis, M. Speletas, Genetics of hereditary angioedema revisited, Clin. Rev. Allergy Immunol. 51 (2016) 170–182 https://doi.org/10.1007/s12016-016-8543-x.

[23] J. Nussberger, M. Cugno, M. Cicardi, A. Agostoni, Local bradykinin generation in hereditary angioedema, J. Allergy Clin. Immunol. 104 (1999) 1321–1322.

[24] M. Cicardi, A. Banerji, F. Bracho, A. Malbrán, B. Rosenkranz, M. Riedl, K. Bork, W. Lumry, W. Aberer, H. Bier, M. Bas, J. Greve, T.K. Hoffmann, H. Farkas, A. Reshef, B. Ritchie, S.W. Yang, J. Grabbe, S. Kivity, W. Kreuz, R.J. Levy, T. Luger, K. Obtulowicz, P. Schmid-Grendelmeier, C. Bull, B. Sitkauskiene, W.B. Smith, E. Toubi, S. Werner, S. Anné, J. Björkander, L. Bouillet, E. Cillari, D. Hurewitz, K.W. Jacobson, C.H. Katelaris, M. Maurer, H. Merk, J.A. Bernstein, C. Feighery, B. Floccard, G. Gleich, J. Hébert, M. Kaatz, P. Keith, C.H. Kirkpatrick, D. Langton, L. Martin, C. Pichler, D. Resnick, D. Wombolt, D.S. Fernández Romero, A. Zanichelli, F. Arcoleo, J. Knolle, I. Kravec, L. Dong, J. Zimmermann, K. Rosen, W.T. Fan, Icatibant, a new bradykinin-receptor antagonist, in hereditary angioedema, N. Engl. J. Med. 363 (2010) 532–541 https://doi.org/10.1056/NEJMoa0906393.

[25] F. Bossi, F. Fischetti, D. Regoli, P. Durigutto, B. Frossi, F. Gobeil Jr., B. Ghebrehiwet, E.I. Peerschke, M. Cicardi, F. Tedesco, Novel pathogenic mechanism and therapeutic approaches to angioedema associated with C1 inhibitor deficiency, J. Allergy Clin. Immunol. 124 (2009) 1303–1310 https://doi.org/10.1016/j.jaci.2009.08.007.

[26] J. Nussberger, M. Cugno, C. Amstutz, M. Cicardi, A. Pellacani, A. Agostoni, Plasma bradykinin in angio-oedema, Lancet 351 (1998) 1693–1697 https://doi.org/10.1016/S0140-6736(97)09137-X.

[27] S. Caccia, C. Suffritti, T. Carzaniga, R. Berardelli, S. Berra, V. Martorana, A. Fra, C. Drouet, M. Cicardi, Intermittent C1-inhibitor deficiency associated with recessive inheritance: functional and structural insight, Sci. Rep. 8 (2018) 977 https://doi.org/10.1038/s41598-017-16667-w.

[28] E. Pappalardo, S. Caccia, C. Suffritti, A. Tordai, L.C. Zingale, M. Cicardi, Mutation screening of C1 inhibitor gene in 108 unrelated families with hereditary angioedema: functional and structural correlates, Mol. Immunol. 45 (45) (2008) 3536–3544 https://doi.org/10.1016/j.molimm.2008.05.007.

[29] M. Speletas, A. Szilagyi, F. Psarros, D. Moldovan, M. Magerl, M. Kompoti, E. Gramoustianou, A. Bors, E. Mihaly, A. Tordai, A. Avramouli, L. Varga, M. Maurer, H. Farkas, A.E. Germenis, Hereditary angioedema: molecular and clinical differences among European populations, J. Allergy Clin. Immunol. 135 (2015) 570–573

https://doi.org/10.1111/all.12714.
[30] G. Loules, M. Zamanakou, F. Parsopoulou, S. Vatsiou, F. Psarros, D. Csuka, G. Porebski, K. Obtulowicz, A. Valerieva, M. Staevska, A. López-Lera, M. López-Trascasa, D. Moldovan, M. Magerl, M. Maurer, M. Speletas, H. Farkas, A.E. Germenis, Targeted next-generation sequencing for the molecular diagnosis of hereditary angioedema due to C1-inhibitor deficiency, Gene 667 (2018) 76–82 https://doi.org/10.1016/j.gene.2018.05.029.
[31] S. Vatsiou, M. Zamanakou, F. Psarros, M. Speletas, G. Loules, F. Parsopoulou, A. Valerieva, H. Farkas, A.E. Germenis, A deep intronic SERPING1 variant associated with hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE), Allergy Asthma Clin. Immunol. (2019).
[32] G. Dewald, K. Bork, Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor, Biochem. Biophys. Res. Commun. 343 (2006) 1286–1289 https://doi.org/10.1016/j.bbrc.2006.03.092.
[33] K. Bork, K. Wulff, P. Meinke, N. Wagner, J. Hardt, G. Witzke, A novel mutation in the coagulation factor 12 gene in subjects with hereditary angioedema and normal C1-inhibitor, Clin. Immunol. 141 (2011) 31–35 https://doi.org/10.1016/j.clim.2011.07.002.
[34] N. Kiss, E. Barabás, K. Várnai, A. Halász A, L.Á. Varga, Z. Prohászka, H. Farkas, Á. Szilágyi, Novel duplication in the F12 gene in a patient with recurrent angioedema, Clin. Immunol. 149 (2013) 142–145 https://doi.org/10.1016/j.clim.2013.08.001.
[35] K. Bork, K. Wulff, G. Witzke, J. Hardt, Hereditary angioedema with normal C1-INH with versus without specific F12 gene mutations, Allergy 70 (2015) 1004–1012 https://doi.org/10.1111/all.12648.
[36] J. Björkqvist, S. de Maat, U. Lewandrowski, A. Di Gennaro, C. Oschatz, K. Schönig, M.M. Nöthen, C. Drouet, H. Braley, M.W. Nolte, A. Sickmann, C. Panousis, C. Maas, T. Renné, Defective glycosylation of coagulation factor XII underlies hereditary angioedema type III, J. Clin. Investig. 125 (2015) 3132–3146 https://doi.org/10.1172/JCI77139.
[37] S. de Maat, C. Maas, Factor XII: form determines function, J. Thromb. Haemost. 14 (2016) 1498–1506 https://doi.org/10.1111/jth.13383.
[38] S. de Maat, J. Björkqvist, C. Suffritti, C.P. Wiesenekker, W. Nagtegaal, A. Koekman, S. van Dooremalen, G. Pasterkamp, P.G. de Groot, M. Cicardi, T. Renné, C. Maas, Plasmin is a natural trigger for bradykinin production in patients with hereditary angioedema with factor XII mutations, J. Allergy Clin. Immunol. 138 (2016) 1414–1423 e9 https://doi.org/10.1016/j.jaci.2016.02.021.
[39] I. Ivanov, A. Matafonov, M.F. Sun, B.M. Mohammed, Q. Cheng, S.K. Dickeson, S. Kundu, I.M. Verhamme, A. Gruber, K. McCrae, D. Gailani, A mechanism for hereditary angioedema with normal C1 inhibitor: an inhibitory regulatory role for the factor XII heavy chain, Blood 133 (2019) 1152–1163 https://doi.org/10.1182/blood-2018-06-860270.
[40] K. Bork, K. Wulff, G. Witzke, J. Hardt, Hereditary angioedema with normal C1-INH with versus without specific F12 gene mutations, Allergy 70 (2015) 1004–1012 https://doi.org/10.1111/all.12648.
[41] M. Magerl, A.E. Germenis, C. Maas, M. Maurer, HAE with normal C1INH: update on evaluation and treatment, Immunol. Allergy Clin. N. Am. 37 (2017) 571–584 https://doi.org/10.1016/j.iac.2017.04.004.
[42] M. Zamanakou, G. Loules, D. Csuka, M. Bova, F. Psarros, F. Parsopoulou, M. Speletas, D. Firinu, T. De Pasquale, A. Zoli, A. Radice, S. Pizzimenti, A. Zanichelli, E. Manoussakis, G.N. Konstantinou, H. Farkas, M. Cicardi, A.E. Germenis, Genotyping unknown hereditary angioedema (U-HAE) and idiopathic non-histaminergic acquired angioedema (Inh-AAE): preliminary evidence of pathogenic variants, Allergy Asthma Clin. Immunol. 13 (Suppl 2) (2017) 29 https://doi.org/10.1186/s13223-017-0198-5.
[43] G. Dewald, A missense mutation in the plasminogen gene, within the plasminogen kringle 3 domain, in hereditary angioedema with normal C1 inhibitor, Biochem. Biophys. Res. Commun. 498 (2018) 193–198 https://doi.org/10.1016/j.bbrc.2017.12.060.
[44] G. Loules, F. Parsopoulou, M. Zamanakou, D. Csuka, M. Bova, T. González-Quevedo, F. Psarros, G. Porebski, M. Speletas, D. Firinu, T. De Pasquale, A. Zoli, A. Radice, S. Pizzimenti, C. Suffritti, E. Manoussakis, M. Cicardi, H. Farkas, A.E. Germenis, Variant pathogenicity curation in primary angioedema with normal C1 inhibitor (nl-C1-INH-HAE), Allergy Asthma Clin. Immunol. (2019).
[45] A.E. Germenis, G. Loules, M. Zamanakou, F. Psarros, T. González-Quevedo, M. Speletas, K. Bork, K. Wulff, L. Steinmüller-Magin, I. Braenne, P. Staubach-Renz, G. Witzke, J. Hardt, On the pathogenicity of the plasminogen K330E mutation for hereditary angioedema, Allergy 73 (2018) 1751–1753 https://doi.org/10.1111/all.13324.
[46] A. Belbézier, G. Hardy, R. Marlu, F. Defendi, C. Dumestre Perard, I. Boccon-Gibod, D. Launay, L. Bouillet, Plasminogen gene mutation with normal C1 inhibitor hereditary angioedema: three additional French families, Allergy 73 (2018) 2237–2239 https://doi.org/10.1111/all.13543.
[47] A. Recke, E.G. Massalme, U. Jappe, L. Steinmüller-Magin, J. Schmidt, Y. Hellenbroich, I. Hüning, G. Gillessen-Kaesbach, D. Zillikens, K. Hartmann, Identification of the recently described plasminogen gene mutation p.Lys330Glu in a family from Northern Germany with hereditary angioedema, Clin. Transl. Allergy 9 (2019) 9 https://doi.org/10.1186/s13601-019-0247-x.
[48] T. Fields, B. Ghebrehiwet, A.P. Kaplan, Kinin formation in hereditary angioedema plasma: evidence against kinin derivation from C2 and in support of "spontaneous" formation of bradykinin, J. Allergy Clin. Immunol. 72 (1983) 54–60.
[49] J. Nussberger, M. Cugno, C. Amstutz, M. Cicardi, A. Pellacani, A. Agostoni, Plasma bradykinin in angio-oedema, Lancet 351 (1998) 1693–1697 https://doi.org/10.1016/S0140-6736(97)09137-X.
[50] A. Pellacani, H.R. Brunner, J. Nussberger, Plasma kinins increase after angiotensin-converting enzyme inhibition in human subjects, Clin. Sci. 87 (1994) 567–574.
[51] M. Cugno, M. Cicardi, B. Bottasso, R. Coppola, R. Paonessa, P.M. Mannucci, A. Agostoni, Activation of the coagulation cascade in C1-inhibitor deficiencies, Blood 89 (1997) 3213–3218.
[52] C. Suffritti, A. Zanichelli, L. Maggioni, E. Bonanni, M. Cugno, M. Cicardi, High-molecular-weight kininogen cleavage correlates with disease states in the bradykinin-mediated angioedema due to hereditary C1-inhibitor deficiency, Clin. Exp. Allergy 44 (2014) 1503–1514 https://doi.org/10.1111/cea.12293.
[53] Z.L.M. Hofman, S. de Maat, C. Suffritti, A. Zanichelli, C. van Doorn, S.A.E. Sebastian, N. Veszeli, D. Csuka, T. Renné, G. Pasterkamp, M. Cicardi, H. Farkas, C.E. Hack, C. Maas, Cleaved kininogen as a biomarker for bradykinin release in hereditary angioedema, J. Allergy Clin. Immunol. 140 (2017) 1700–1703 e8 https://doi.org/10.1016/j.jaci.2017.07.012.
[54] A. Banerji, P. Busse, M. Shennak, W. Lumry, M. Davis-Lorton, H.J. Wedner, J. Jacobs, J. Baker, J.A. Bernstein, R. Lockey, H.H. Li, T. Craig, M. Cicardi, M. Riedl, A. Al-Ghazawi, C. Soo, R. Iarrobino, D.J. Sexton, C. TenHoor, J.A. Kenniston, R. Faucette, J.G. Still, H. Kushner, R. Mensah, C. Stevens, J.C. Biedenkapp, Y. Chyung, B. Adelman, Inhibiting plasma kallikrein for hereditary angioedema prophylaxis, N. Engl. J. Med. 376 (2017) 717–728 https://doi.org/10.1056/NEJMoa1605767.
[55] M.L. Lara-Marquez, S.C. Christiansen, M.A. Riedl, J. Herschbach, B.L. Zuraw, Threshold-stimulated kallikrein activity distinguishes bradykinin- from histamine-mediated angioedema, Clin. Exp. Allergy 48 (2018) 1429–1438 https://doi.org/10.1111/cea.13219.
[56] A. Deroux, I. Vilgrain, C. Dumestre-Pérard, I. Boccon-Gibod, L. Bouillet, Towards a specific marker for acute bradykinin-mediated angioedema attacks: a literature review, Eur. J. Dermatol. 25 (2015) 290–295 https://doi.org/10.1684/ejd.2015.2547.
[57] H. Farkas, N. Veszeli, E. Kajdácsi, L. Cervenak, L. Varga, "Nuts and bolts" of laboratory evaluation of angioedema, Clin. Rev. Allergy Immunol. 51 (2016) 140–151 https://doi.org/10.1007/s12016-016-8539-6.
[58] S.C. Christiansen, B.L. Zuraw, Laboratory approaches for assessing contact system activation, Immunol. Allergy Clin. N. Am. 37 (2017) 527–539 http://doi.org/10.1016/j.iac.2017.04.008.
[59] A.P. Kaplan, C. Maas, The search for biomarkers in hereditary angioedema, Front. Med. 4 (2017) 206 https://doi.org/10.3389/fmed.2017.00206.
[60] M. Speletas, A. Szilágyi, D. Csuka, N. Koutsostathis, F. Psarros, D. Moldovan, M. Magerl, M. Kompoti, L. Varga, M. Maurer, H. Farkas, A.E. Germenis, *F12*-46C/T polymorphism as modifier of the clinical phenotype of hereditary angioedema, Allergy 70 (2015) 1661–1664 https://doi.org/10.1111/all.12714.
[61] P. Gianni, G. Loules, M. Zamanakou, M. Kompoti, D. Csuka, F. Psarros, M. Magerl, D. Moldovan, M. Maurer, M.G. Speletas, H. Farkas, A.E. Germenis, Genetic determinants of the C1-INH deficiency angioedema (C1-INH-HAE) age of onset, Int. J. Allergy Immunol. 174 (2017) 200–204 https://doi.org/10.1159/000481987.
[62] T. Hoover, M. Lippmann, E. Grouzmann, F. Marceau, P. Herscu, Angiotensin converting enzyme inhibitor induced angio-oedema: a review of the pathophysiology and risk factors, Clin. Exp. Allergy 40 (2010) 50–61 https://doi.org/10.1111/j.1365-2222.2009.03323.x.
[63] G. Pare, M. Kubo, J.B. Byrd, C.A. McCarty, A. Woodard-Grice, K.K. Teo, S.S. Anand, R.L. Zuvich, Y. Bradford, S. Ross, Y. Nakamura, M. Ritchie, N.J. Brown, Genetic variants associated with angiotensin-converting enzyme inhibitor-associated angioedema, Pharmacogenetics Genom. 23 (2013) 470–478 https://doi.org/10.1097/FPC.0b013e328363c137.
[64] B.P. Roques, F. Noble, V. Dauge, M.C. Fournie-Zaluski, A. Beaumont, Neutral endopeptidase 24.11: structure, inhibition, and experimental and clinical pharmacology, Pharmacol. Rev. 45 (1993) 87–146.
[65] E.H. Howell, S.J. Cameron, Neprilysin inhibition: a brief review of past pharmacological strategies for heart failure treatment and future directions, Cardiol. J. 23 (2016) 591–598 https://doi.org/10.5603/CJ.a2016.0063.
[66] A. Ferlini, C. Scotton, G. Novelli, Biomarkers in rare diseases, Public Health Genomics 16 (2013) 313–321 https://doi.org/10.1159/000355938.
[67] M.K. Vernon, A.M. Rentz, K.W. Wyrwich, M.V. White, A. Grienenberger, Psychometric validation of two patient-reported outcome measures to assess symptom severity and changes in symptoms in hereditary angioedema, Qual. Life Res. 18 (2009) 929–939 https://doi.org/10.1007/s11136-009-9509-8.
[68] C.V. McMillan, J. Speight, A. Relan, L. Bellizzi, G. Haase, M. Cicardi, Content validity of visual analog scales to assess symptom severity of acute angioedema attacks in adults with hereditary angioedema: an interview study, Patient 5 (2012) 113–126 https://doi.org/10.2165/11597490-000000000-00000.
[69] G. Appelboom, M. LoPresti, J.Y. Reginster, E. Sander Connolly, E.P. Dumont, The quantified patient: a patient participatory culture, Curr. Med. Res. Opin. 30 (2014) 2585–2587 https://doi.org/10.1185/03007995.2014.954032.
[70] R. Schnall, M. Rojas, S. Bakken, W. Brown, A. Carballo-Dieguez, M. Carry, D. Gelaude, J.P. Mosley, J. Travers, A user-centered model for designing consumer mobile health (mHealth) applications (apps), J. Biomed. Inform. 60 (60) (2016) 243–251 https://doi.org/10.1016/j.jbi.2016.02.002.
[71] L. Hood, S.H. Friend, Predictive, personalized, preventive, participatory (P4) cancer medicine, Nat. Rev. Clin. Oncol. 8 (2011) 184–187 https://doi.org/10.1038/nrclinonc.2010.227.
[72] J.T. Dudley, J. Listgarten, O. Stegle, S.E. Brenner, L. Parts, Personalized medicine: from genotypes, molecular phenotypes and the quantified self, towards improved medicine, Pac. Symp. Biocomput. (2015) 342–346 https://doi.org/10.1142/9789814644730_0033.
[73] F. Perego, The International Registry for Angioedema without Wheals, EAACI Helsinki, 2017.
[74] L. Abrahamyan, B.M. Feldman, G. Tomlinson, M.E. Faughnan, S.R. Johnson,

I.R. Diamond, S. Gupta, Alternative designs for clinical trials in rare diseases, Am. J. Med. Genet. Part C Semin. Med. Genet. 172 (2016) 313–331 https://doi.org/10.1002/ajmg.c.31533.

[75] W.A. Demeyin, J. Frost, O.C. Ukoumunne, S. Briscoe, N. Britten, N of 1 trials and the optimal individualisation of drug treatments: a systematic review protocol, Syst. Rev. 6 (2017) 90 https://doi.org/10.1186/s13643-017-0479-6.

[76] OCEBM Levels of Evidence Working Group, The Oxford levels of evidence 2. Oxford Centre for evidence-based medicine, https://www.cebm.net/index.aspx?o=5653.

[77] H. Longhurst, M. Cicardi, T. Craig, K. Bork, C. Grattan, J. Baker, H.H. Li, A. Reshef, J. Bonner, J.A. Bernstein, J. Anderson, W.R. Lumry, H. Farkas, C.H. Katelaris, G.L. Sussman, J. Jacobs, M. Riedl, M.E. Manning, J. Hebert, P.K. Keith, S. Kivity, S. Neri, D.S. Levy, M.L. Baeza, R. Nathan, L.B. Schwartz, T. Caballero, W. Yang, I. Crisan, M.D. Hernandez, I. Hussain, M. Tarzi, B. Ritchie, P. Králíčková, M. Guilarte, S.M. Rehman, A. Banerji, R.G. Gower, D. Bensen-Kennedy, J. Edelman, H. Feuersenger, J.P. Lawo, T. Machnig, D. Pawaskar, I. Pragst, B.L. Zuraw, COMPACT Investigators, Prevention of hereditary angioedema attacks with a subcutaneous C1 Inhibitor, N. Engl. J. Med. 376 (2017) 1131–1140 https://doi.org/10.1056/NEJMoa1613627.

[78] W.R. Lumry, H. Farkas, D. Moldovan, E. Toubi, J. Baptista, T. Craig, M. Riedl, Icatibant for multiple hereditary angioedema attacks across the controlled and open-label extension phases of FAST-3, Int. Arch. Allergy Immunol. 168 (2015) 44–55 https://doi.org/10.1159/000441060.

[79] H. Farkas, A. Reshef, W. Aberer, T. Caballero, L. McCarthy, J. Hao, W. Nothaft, J. Schranz, J.A. Bernstein, H.H. Li, Treatment effect and safety of icatibant in pediatric patients with hereditary angioedema, J. Allergy Clin. Immunol. Pract. 5 (2017) 1671–1678 https://doi.org/10.1016/j.jaip.2017.04.010.

[80] E.O. Lillie, B. Patay, J. Diamant, B. Issell, E.J. Topol, N.J. Schork, The n-of-1 clinical trial: the ultimate strategy for individualizing medicine? Per. Med. 8 (2011) 161–173 https://doi.org/10.2217/pme.11.7.

[81] M. Rijavec, M. Košnik, S. Andrejević, L. Karadža-Lapić, V. Grivčeva-Panovska, P. Korošec, The functional promoter F12-46C/T variant predicts the asymptomatic phenotype of C1-INH-HAE, Clin. Exp. Allergy (2019), https://doi.org/10.1111/cea.13470 In press.