Investigaton of plasma enzyme systems and neutrophil granulocytes in hereditary angioedema due to C1-inhibitor deficiency

Doctoral Thesis

Nóra Veszeli

Semmelweis University School of Ph. D. studies
Doctoral School of Basic & Translational Medicine

Supervisors: Henriette Farkas MD, DSc
Lilian Varga PhD

Official reviewers: Gabriella Sármay Dsc
Magdolna Krasznai MD, PhD

Chair of the Final Examination Committee:
Edit Buzás MD, DSc

Members of the Final Examination Committee:
Anna Erdei DSc
Erzsébet Komorowicz MD, PhD

Budapest
2018

1. Introduction

1.1 Hereditary angioedema due to the deficiency of the C1-inhibitor

Hereditary angioedema due to the deficiency of the C1-inhibitor (C1-INH) is an autosomal dominant disorder (C1-INH-HAE); its estimated prevalence is one case per 10,000 to 50,000 persons. It is characterized by the episodic recurrence of angioedema in the subcutis and/or in the submucosa. Subcutaneous edema most commonly occurs on the extremities, trunk, face, or genitals, and it is not accompanied by pain, pruritus, or urticaria. Among the submucosal types, edema of the gastrointestinal tract is often accompanied by colicky abdominal pain, nausea, and vomiting. Edema of the upper airways is less common; however, it may lead to life-threatening obstruction, and cause suffocation. When it is not an immediate threat to life, edema formation usually resolves on its own, within 2 to 5 days. The time of the first onset of edematous symptoms, their frequency and severity vary among patients, as well as exhibit considerable inconsistency even within the same family and individual.

In the majority of patients, edematous symptoms are often preceded by prodromal signs. The most specific, objective prodromal symptom is the occurrence of a type of skin rash, known as erythema marginatum. The underlying cause of the disease is the heterozygous deficiency of C1-INH, a heavily glycosylated glycoprotein (consisting of 478 amino acids), which belongs to the serpin (*ser*ine *p*rotease *in*hibitor) superfamily. Two types of C1-INH-HAE are distinguished; both are characterized by reduced C1-INH functional activity. In type I, a mutation in the serpin gene eliminates the production of C1-INH and hence, the blood level of the latter is reduced. In type II, by contrast, the normal and a non-functional, mutant protein are produced simultaneously.

In co-operation with other inhibitors, C1-INH can suppress the activity of certain serin proteases of the complement, coagulation, fibrinolytic, and contact-

kinin systems. This prevents their activation, along with the release of mediators, which exert pro-inflammatory actions and increase vascular permeability.

According to current knowledge, the increase of vascular permeability (resulting from enhanced bradykinin release) underlies the onset of edematous attacks; however, the exact biological background of the evolution and course of edematous attacks is yet unknown. Studies into the pathomechanism of this type of edema formation are based on the comparison of values measured in blood samples obtained from C1-INH-HAE patients on a single occasion, during symptom-free periods, as well as during edematous attacks. In the literature, a number of controversial findings have been published on the pathomechanism. This is possibly explained by the fact that in the majority of cases, conclusions have been drawn from the findings of very small-scale studies (conducted on 5 to 10 patients). Often, the blood samples drawn during edematous episodes or symptom-free periods and used for comparison were not obtained from the same patient. Additional information may be gleaned by complete kinetic monitoring the changes of the individual laboratory parameters – that is, starting in the symptom-free state preceding the edematous attack, and continuing until the complete resolution of the edema.

The objective of my dissertation was to gain a better insight into the pathomechanism of edematous attacks. In particular, the contributions of the involved plasma enzyme systems to the evolution and/or the course of edematous manifestations of C1-INH deficiency have not been investigated or have been only scarcely studied to date.

1.2. The relationship between the complement system and the pathomechanism of edematous attacks in C1-INH-HAE

The findings published in the literature on the functional activity of C1-INH are controversial: increases and decreases during edematous attacks have both been

described. Earlier, our work group found that in treatment-naïve patients, functional activity correlates with the severity of the disease at the time when C1-INH deficiency is diagnosed, and may predict the expected frequency of edematous attacks. Similarly, we have observed that the serum level of the C1/C1-INH complex may predict the expected severity of the disease. Further, as C1/C1-INH complex level increased during edematous attacks compared with symptom-free periods, this parameter, might prove a sensitive marker of early complement activation. CSUKA *et al* found increased MASP-2 concentration, and elevated levels of the ficolin-3/MASP-2 complex during edematous episodes – this suggests activation of the ficolin-lectin pathway. C3 and C5a levels were similar in blood samples drawn during symptom-free periods or during edematous episodes. However, elevated TCC levels and reduced ficolin-3-mediate TCC deposition were found during edematous attacks.

1.3. Relationships between the coagulation and the fibrinolytic systems in the pathomechanism of the edematous episodes of C1-INH-HAE

The findings available so far on the coagulation and on the fibrinolytic systems have been contributed in general by small-scale, non self-controlled studies. Elevated levels of Factor VII – an enzyme of prime importance in the coagulation process – have been observed during edematous attacks of HAE, compared with symptom-free periods. Similarly, prothrombin fragments 1+2 (F1+2) levels (the indices of thrombin generation), were elevated during edematous attacks, and higher than normal levels of the thrombin-antithrombin (TAT) complex were described. Although Factor XI (FXI) levels have never been measured during edematous attacks, a comparison of blood samples obtained from symptom-free C1-INH-HAE patients and from healthy controls did not reveal any difference. In addition to the activation of the coagulation system, the elevated levels of the plasmin-antiplasmin complex, and of the D-dimer suggest the simultaneous activation of the fibrinolytic system during edematous attacks. Notwithstanding

the foregoing, a propensity for thromboembolism has never been described in C1-INH-HAE patients. Remarkably, CUGNO *et al* did not confirm any significant elevation of D-dimer levels during edematous attacks.

1.4. Relationships between neutrophil granulocytes and the pathomechanism of edematous episodes in C1-INH-HAE

Previously, a number of case studies reported the elevation of white blood cell and neutrophil granulocyte counts during edematous attacks.

In 2010, our work group published confirmatory findings on the elevation of WBC and neutrophil cell counts in 18 C1-INH-HAE patients. Further, we showed that the increase in neutrophil cell count was greater than that attributable to hemoconcentration.

The possible role of neutrophil granulocytes in the pathomechanism of C1-INH-HAE has never been investigated – notwithstanding the multiple links between the functions of these cells and the pathophysiology of edematous attacks. In addition to cytokines and bacterial products, the activation of neutrophil granulocytes may be induced – and regulated – by certain components of the plasma enzyme systems, such as PK, C3a, C5a, and Factor H. Neutrophil elastase (NE) released during the activation of these cells can cleave and inactivate C1-INH. This may further enhance the malfunctioning of the regulatory mechanisms, and thereby contribute to the evolution of edematous attacks. Moreover, the activation of these cells may result in the formation of neutrophil extracellular traps (NETs), and the negative charge of the surface of the latter makes activation of the contact-kinin system possible. Besides, it has been reported that the release of kinines (bradykinin, kallidin) may occur also on the surface of neutrophil granulocytes by way of FXII-mediated activation, or the release of tissue kallikrein from neutrophil granulocytes.

All these findings suggest a possible role for an active process involving neutrophil granulocytes in the background of the pathogenesis of the edematous

attacks occurring in C1-INH-HAE. This possibility was studied in the first place.

2. Objective

2.1. Comprehensive, ‘real-time’ investigation of the changes in the coagulation, fibrinolytic, and contact systems in C1-INH-HAE

Earlier studies attempted to interpret the changes observed in various plasma enzyme systems during edematous attacks by analyzing each system separately. The value of their findings are therefore of limited – on one hand because of the small number of study subjects, and on the other hand because the blood samples obtained during symptom-free periods or during attacks were not necessarily drawn from the same patients.

We expanded the range of the parameters investigated in earlier studies – including FXIIa, F1+2, TAT complex, D-dimer, prothrombin time (PTT), with hitherto unstudied parameters, such as Factor XI, fibrinogen, plasminogen, and activated partial thromboplastin time (aPTT), in order to answer the following questions:

1. Compared with the symptom-free period, is there any change in the functioning of the plasma enzyme systems during an edematous attack of C1-INH-HAE?

Simultaneous investigation into these plasma enzyme systems might shed light on the following:

2. What correlations exist within and among the individual plasma enzyme systems with regard to specific parameters during symptom-free periods or during edematous attacks?

Because edematous episodes exhibit substantial variability as regards their location, as well as attacks with multiple location are common, we raised the following question:

3. Is there any difference among the levels of the study parameters, with regard to the various locations of edema formation, or edematous episodes involving a single site or multiple locations? What inter- or intra-individual

differences can be distinguished among the parameters concerning edematous attacks?

Thanks to the volunteering of a patient who had been experiencing recurrent attacks, we got an opportunity to monitor, for the first time, the course of a spontaneously occurring edematous attack, starting from the symptom-free state preceding the attack, until the complete resolution of the symptoms. This observation raised the following important questions:

4. Do the changes occurring in the plasma enzyme systems precede the onset of the edematous episode or coincide with them?
5. What correlations can be identified between the changes of the individual parameters and the clinical symptoms?

2.2. The role of neutrophil granulocytes in C1-INH-HAE

Our investigations explored, for the first time, the presumed role of neutrophil granulocytes in the pathomechanism of C1-INH-HAE. In particular, our work group has detected higher neutrophil granulocyte counts during edematous attacks. As a first approximation, we sought answer to the following question, by studying a larger number of cases:

1. Is the observed increase of neutrophil granulocyte count a real change – in view of the extent of hemoconcentration?

Further, by investigating the biochemical markers released during the activation of neutrophils (NE, myeloperoxidase (MPO), and pentraxin 3 (PTX3)), as well as cytokines (IL-8, TNF-α), complement factors (Factor H, C5a), and CRP acting on the latter process, we raised the following questions:

2. Do neutrophil granulocytes undergo activation during the edematous attacks of C1-INH-HAE?
3. What correlations exist among the measured parameters during symptom-free periods and during edematous attacks?

During the complete kinetic follow-up of an edematous attack of a C1-INH-HAE patient, by determining neutrophil granulocyte count and neutrophil activation markers we raised the following question:

4. Do the activation of neutrophil cells and the increase of their numbers precede the onset of the edematous attacks, or occur concomitantly?

2.3. The immediate consequences of C1-INH deficiency during the course of an edematous episode of C1-INH-HAE

Although a relationship between C1-INH level and clinical symptoms might appear straightforward in the context of a disorder with C1-INH deficiency, this has been investigated by a few studies only. During the follow-up of an edematous attack, we compared the changes of C1-INH level and those of clinical manifestations, in order to answer the following question:

1. Is there any correlation between C1-INH level and the onset or the resolution of edematous manifestations?

Because C1-INH is the exclusive regulator of the serin proteases C1r and C1s, we analyzed the consequences of C1-INH deficiency by studying the parameters of the classical pathway of complement system (C1q, C1s, C4, and C4a).

2. Can the activation of the classical pathway of complement be demonstrated?

By investigating C3a activation products generated during the cleavage of C3 components (which have a central role in the complement system), as well as by studying the activation products of the terminal pathway (C5a, TCC), we intended to answer the following question:

3. Do the effects of C1-INH deficiency influence the cleavage of the C3 component, and the subsequent steps of the activation of the complement cascade?

3. Methods

3.1. Patients and control subjects

The study subjects were recruited from the patient population managed at the Hungarian Angioedema Center between 2008 and 2005. We included patients in whom blood sampling was feasible also during edematous attacks. We analyzed 87 blood samples drawn during edematous attacks, from 43 patients. My dissertation is based on three studies:

1) The investigation of plasma enzyme systems in C1-INH-HAE;
2) The investigation of neutrophil granulocytes in C1-INH-HAE;
3) 'Real-time' monitoring of the course of an edematous attack in a patient with C1-INH-HAE.

Because of the overlaps among the groups of enrolled patients, the properties of the populations of the three studies will be described separately, as follows:

3.1.1 The investigation of plasma enzyme systems in C1-INH-HAE patients and in healthy controls

Thirty-nine patients (12 males and 27 females with a median age of 35 years; 33 with type I and 6 with type II C1-INH-HAE), and 40 healthy controls (15 males and 25 females with a median age of 33 years) were enrolled. In total, we analyzed 62 blood samples obtained during edematous episodes. At least one sample (but maximum five), drawn during edematous attacks, was available from each patient. Twenty-one of these samples were obtained during an episode of submucosal (19 abdominal and 2 upper airway) edema; 29 were drawn during subcutaneous attacks; and 12 during episodes with edema formation in "mixed" (subcutaneous and submucosal) locations.

3.1.2. The investigation of neutrophil granulocytes in C1-INH-HAE patients and in healthy controls

Twenty-six patients (6 males and 20 females with a median age of 33 years; 16 patients had type I and 7 had type II C1-INH-HAE), and 26 healthy controls (7 males and 19 females with a median age of 37 years) were included. We analyzed 26 blood samples obtained during an edematous attack (i.e. one sample from each study subject).

3.1.3. Complete kinetic follow-up of the course of an edematous episode in a C1-INH-HAE patient with the collection of parallel blood samples from a healthy control subject

In a 56-year-old female patient with type I C1-INH-HAE, we performed – for the first time – complete kinetic foloww-up of an edematous episode by means of the continuous recording of symptoms, and by periodic blood sampling. Monitoring started in the symptom-free period preceding the attack, and continued until the complete resolution of edema. During the whole duration of this study, the patient recorded her symptoms in a diary, along with the times of their onset and resolution. Symptoms severity was graded and documented using a 0-100 mm visual analogue scale (VAS), separately for the prodromal and for the edematous manifestations. In total, 12 blood samples were obtained during the period of monitoring.

A 52-year-old, healthy, female volunteer served as control; she had no known disease, and did not receive medicinal products during this study.

3.2 Laboratory methods

White blood cell and neutrophil granulocyte counts, as well as hematocrit values were determined from EDTA-anticoagulated whole blood, with an Advia 120 Hematology System (Siemens Healthineers, Erlangen, Germany).

Serum, EDTA-, or citrate-plasma samples reserved for further analyses were stored at minus 70 °C temperature until processing.

In serum samples from patients and from healthy controls, C1q, Factor H levels, and C1-INH activity were determined with ELISA. C1r level was measured with radial immunodiffusion assay, whereas an automate chemistry analyzer was used to determine C3, C4, and CRP levels.

EDTA-plasma samples from patients and healthy controls were used to determine the concentrations of C1-INH, C1s, C3a, C4a, C5a, TCC, Factor H, NE, MPO, PTX3, IL-8, and TNF-α with ELISA.

In citrated plasma samples from patients and healthy controls, the levels of activated FXI and FXII were determined with coagulometry, whereas standard laboratory methods were applied to measure prothrombin time and activated partial thromboplastin time. Fibrinogen level was determined with the Clauss method. Plasminogen, plasminogen activator inhibitor 1, and D-dimer levels were measured with an automated laboratory system. The levels of F1+2 and of the TAT complex were determined with ELISA.

3.3. Statistical analysis

Statistical analysis was performed and graphs were plotted using the Prism 5 for Windows software package (GraphPad Software, San Diego, USA). All statistical tests were considered significant at a probability level of $p<0.05$.

4. Results

4.1. Simultaneous, comprehensive evaluation of the changes of the coagulation, fibrinolytic, and contact systems in C1-INH-HAE

Compared with the blood samples obtained from the same patients during symptom-free periods, FXIIa, FXIa, F1+2, and TAT-complex levels were significantly higher in samples drawn during edematous attacks.

We studied – for the first time – the changes of PT and of aPTT measured in symptom-free periods, and during attacks. PT values determined in symptom-free periods and in healthy controls were similar, whereas PT and aPTT were significantly shorter during edematous attacks than during symptom-free periods. Furthermore aPTT were shorter during symptom-free period than in healthy controls.

Compared with healthy controls, D-dimer levels were elevated in the samples of C1-INH-HAE patients. Moreover, we detected further increase during edematous attacks, in comparison with symptom-free periods. Plasminogen levels were similar between patients and controls. PAI-1 levels were lower during edematous episodes than during symptom-free periods, or than those determined in healthy controls. We measured higher D-dimer and plasminogen levels, as well as shorter PT values during episodes involving multiple locations, compared with those occurring at a single location. We did not detect any significant difference between the episodes of subcutaneous or submucosal edema formation, with regard to the individual parameters of plasma enzyme systems. During the kinetic follow-up of the natural course of an edematous attack in one of our patients, the onset of edematous symptoms was accompanied by the marked elevation (above the normal range) of F1+2 and of TAT-complex levels. These levels remained high, and started to decrease with symptomatic improvement. D-dimer levels were consistently the same during symptom-free and in prodromal periods; just as these levels stayed normal in the

healthy control subject over the 24-hour period of monitoring. However, starting at the onset of edematous symptoms, D-dimer levels started to increase and then, peaked at a level 100 times higher than at baseline, and started to decline during the resolution of edema.

4.2. The role of neutrophil granulocytes in C1-INH-HAE

Compared with those found in healthy controls, white blood cell and neutrophil granulocyte counts were significantly higher in blood samples obtained from C1-INH-HAE patients during symptom-free periods, and these values increased further during edematous attacks. During the latter, NE, MPO, and PTX3 levels also increased significantly, in comparison to those measured during symptom-free periods, or in healthy controls. However the levels determined in C1-INH-HAE patients during symptom-free periods did not differ from those of healthy controls. In blood samples drawn during edematous attacks, we found a positive correlation of neutrophil granulocyte count with NE and MPO levels, as well as between NE and MPO levels. Neutrophil granulocyte and white blood cell counts exhibited a more marked increase upon the onset of edema, began to decrease with the improvement of edematous symptoms, and returned to baseline by the time of their resolution. The levels of the activation markers NE and MPO fluctuated throughout the entire observation period. PTX3 level reached a definite peak during the edematous attack, but following the resolution of edematous symptoms, its value corresponded to that measured at baseline during the symptom-free period.

4.3. The immediate consequences of C1-INH deficiency during the course of an edematous episode of C1-INH-HAE

C1-INH concentration and functional activity decreased progressively during the entire monitoring period. At the time of the onset of the edematous episode, both

the concentration and the activity of C1-INH decreased by half, compared with the baseline value measured during the symptom-free period. These parameters did not increase during symptomatic improvement, or following the complete resolution of edematous symptoms.

C4 concentration was the highest at the start of monitoring, when the patient was yet symptom-free. Then, it began to decrease upon the occurrence of prodromal symptoms, and declined further with the development of edema. This declining trend did not change, notwithstanding the improvement of edematous symptoms: C4 level reached the lower limit of detection and stayed there for the remainder of the observation period. Subsequently, we could not detect any increase of C4 concentration even after the complete resolution of edematous symptoms. The level of the C4a activation product was elevated during prodromal period, compared with the baseline value measured at the start of monitoring. It peaked at a four times higher maximum in the 7^{th} hour preceding the onset of the edematous episode compared with the baseline value, and this level was seven times higher than that determined in the healthy control subject. C4a level remained high during the edematous episode, then decreased simultaneously with the improvement of symptoms. The levels of the C3a, C5a, and TCC activation products did not show any trend for increase, and throughout the monitoring period, their concentrations remained below those measured in the healthy control subject.

5. Conclusions

The following are the principal results of my work:

1. In a study conducted on a larger number of patients, we confirmed the activation of the contact, coagulation, and fibrinolytic systems during edematous attacks, by comparing blood samples drawn from the same patients during symptom-free periods and during attacks. This assertion is based on elevated FXIIa, F1+2, TAT-complex, and D-dimer levels, as well as on shortened PT and aPTT *(6.1.1)*. In the patients, pro-coagulant and fibrinolytic activity was increased during the symptom-free period. This activity was lower than that seen during edematous episodes, but higher than that observed in healthy controls *(6.1.1)*. Based on the findings of the 'real-time' monitoring of a patient over 96 hours, we conclude that the activation of these systems occurs parallel with the development of edema. This is evidenced by the fact that except for fibrinogen level, all other parameters remained within the range determined in the healthy control subject. *(This result is in the process of being published.*)
2. The properties of the edematous attacks exhibited high inter- and intra-individual variation. This applies not only to visible clinical symptoms and their severity, but also to the activation state of plasma enzyme systems, as well as to the fluctuation of their indices during the periods between consecutive edematous attacks. We studied, for the first time, whether the edematous episodes occurring at a single site or at multiple locations exert any influence on the levels of the parameters of plasma enzyme systems. We found that both the coagulation and the fibrinolytic system undergo more intense activation during edematous episodes involving multiple locations *(6.1.1)*.

3. Our study – conducted on a larger number of cases and in consideration of the extent of hemoconcentration – confirmed that during the edematous episodes of C1-INH-HAE, neutrophil granulocyte count is higher than that measured in the same patients during symptom-free periods *(6.1.2)*. Further, we showed that compared with healthy controls, neutrophil granulocyte count is higher in C1-INH-HAE patients, even when they are symptom-free *(6.1.2)*. Neutrophil granulocyte count does not change previous to the development of edematous attack; however, it increases dramatically upon the onset, and concomitantly with the evolution of the edematous attack. (*This result is in the process of being published.*)

4. By comparing the relevant parameters in the blood samples drawn from the same C1-INH-HAE patients during symptom-free periods and during edematous attacks, we studied, for the first time, whether neutrophil granulocytes undergo activation during these attacks. The elevation of NE, MPO, and PTX3 levels suggested the activation of neutrophil granulocytes during edematous attacks of C1-INH-HAE. This finding is confirmed by the strong correlation detected between neutrophil granulocyte count and NE as well as MPO. Importantly, these correlations could not be observed in blood samples obtained from patients during symptom-free period, or in those from healthy controls *(6.1.2)*.

5. We succeeded, for the first time, the complete kinetic follow-up of the natural course of an edematous attacks of C1-INH-HAE – that is, from its onset, through its evolution, and until its resolution. We found a correlation between the measurable decline of C1-INH level in the systemic circulation, and the onset of edematous symptoms. However, it appears that factors other than C1-INH might have a role in the spontaneous resolution of the edematous attack *(6.1.3)*.

6. The prodromal period of the edematous episodes has never been studied yet. Our study was the first to explore this period during the monitoring of an edematous attack in a patient with C1-INH-HAE. We can conclude that the processes leading to edema formation may begin as early as at the onset of prodromal symptoms. This is suggested by the changes of the parameters of the classical pathway (decrease of C1-INH level, C1 subcomponents, and C4, as well as the elevation of C4a level). Considering these indices, the elevation of C4a level during the prodromal period might serve as a biomarker for the prediction of edematous attacks *(6.1.3)*.
7. The observed decrease of C4 and the elevation of C4a levels indicate activation of the classical pathway of the complement system. On the other hand, the concentrations of the C3a, C5a, and TCC activation products did not change during the observation period, and were lower than in healthy controls. This suggests that activation stops at the C3 step, and no further activation can be observed along the terminal pathway (6.1.3).

6. Bibliography of the Candidate's publications

6.1. Publications related to the PhD thesis

1. Csuka D*, Veszeli N*, Imreh É, Zotter Z, Skopál J, Prohászka Z, Varga L, Farkas H. Comprehensive study into the activation of the plasma enzyme systems during attacks of hereditary angioedema due to C1-inhibitor deficiency.
   Orphanet J Rare Dis. 2015 Oct 9;10:132. IF: 3.290
   * Shared lead authorship

2. Veszeli N, Csuka D, Zotter Z, Imreh É, Józsi M, Benedek S, Varga L, Farkas H.
   Neutrophil activation during attacks in patients with hereditary angioedema due to C1-inhibitor deficiency.
   Orphanet J Rare Dis. 2015 Dec 10;10:156. IF: 3.290

3. Veszeli N, Kőhalmi KV, Kajdácsi E, Gulyás D, Temesszentandrási G, Cervenak L,
   Farkas H, Varga L. Complete kinetic follow-up of symptoms and complement parameters during a hereditary angioedema attack.
   Allergy. DOI: 10.1111/all.13327 (2017) IF: 7.361*
   **The impakt factor of the given year is not available*

Cumulative IF of the publications related to the subject of the PhD thesis: **12.29**

6.2. Other publications

1. Veszeli N, Füst G, Csuka D, Trauninger A, Bors L, Rozsa C, Nagy Z, Jobbágy Z, Eizler K, Prohászka Z, Varga L, Illes Z. A systematic analysis of the complement pathways in patients with neuromyelitis optica indicates alteration but no activation during remission. (2014)
Mol Immunol. 2014 Feb;57(2):200-9. IF:2.973

2. Farkas H, Csuka D, Veszeli N, Zotter Z, Szabó E, Varga L. Home treatment of attacks with conestat alfa in hereditary angioedema due to C1-inhibitor deficiency. (2014)
Allergy Asthma Proc. 2014 May-Jun;35(3):255-9. IF:3.061

3. Zotter Z, Veszeli N, Csuka D, Varga L, Farkas H. Frequency of the virilisingeffects of attenuated androgens reported by women with hereditary angioedema. (2014)

Orphanet J Rare Dis. 2014 Dec 5;9:205. IF:3.358

4. Farkas H, Veszeli N, Csuka D, Temesszentandrási G, Tóth F, Kőszegi L, Varga L.Management of pregnancies in a hereditary angioedema patient after treatment with attenuated androgens since childhood. (2015)
J Obstet Gynaecol. 2015 Jan;35(1):89-90. IF:0.611

5. Farkas H, Kőhalmi KV, Veszeli N, Zotter Z, Várnai K, Varga L. Risk of thromboembolism in patients with hereditary angioedema treated withplasma-derived C1-inhibitor. (2016)
Allergy Asthma Proc. 2016 Mar-Apr;37(2):164-70. IF:2.614

6. Kőhalmi KV, Veszeli N, Zotter Z, Csuka D, Benedek S, Imreh É, Varga L, Farkas H. The effect of long-term danazol treatment on haematological parameters inhereditary angioedema. (2016).
Orphanet J Rare Dis. 2016 Feb 25;11:18. IF:3.478

7. Farkas H, Kőhalmi KV, Veszeli N, Tóth F, Varga L. First report of icatibant treatment in a pregnant patient with hereditary angioedema. (2016).
J Obstet GynaecolRes. 2016 Aug;42(8):1026-8. IF:1.099

8. Farkas H, Veszeli N, Kajdácsi E, Cervenak L, Varga L. "Nuts and Bolts" of Laboratory Evaluation of Angioedema. (2016).
Clin Rev Allergy Immunol. 2016 Oct;51(2):140-51.
The journal IF in 2016 is 5.263, but due to the classification of the publication it is not listed.

9. Veszeli Nóra, Kőhalmi Kinga Viktória. A herediter angioödéma gyermekkori sajátosságai, diagnosztikája és korszerű kezelése.
Gyermekorvos továbbképzés. 2016. XV. évfolyam 2.szám IF:-

10. Czaller I, Csuka D, Zotter Z, Veszeli N, Takács E, Imreh É, Varga L, Farkas H.Thyroid hormones and complement parameters in hereditary angioedema with C1-inhibitor deficiency. (2016).
Ann Allergy Asthma Immunol. 2016 Aug;117(2):175-9. IF:3.728

11. Zotter Z, Veszeli N, Kőhalmi KV, Varga L, Imreh É, Kovács G, Nallbani M,Farkas H. Bacteriuria increases the risk of edematous attacks in hereditaryangioedema with C1-inhibitor deficiency. (2016).
Allergy. 2016 Dec;71(12):1791-1793. IF:7.361

12. Zotter Z, Nagy Z, Patócs A, Csuka D, Veszeli N, Kőhalmi KV, Farkas H. Glucocorticoid receptor gene polymorphisms in hereditary angioedema withC1-inhibitor deficiency. (2017)
Orphanet J Rare Dis. 2017 Jan 10;12(1):5. IF:3.478*
** The impakt factor of the given year is not available*

13. Engel-Yeger B, Farkas H, Kivity S, Veszeli N, Kőhalmi KV, Kessel A. Health-related quality of life among children with hereditary angioedema. (2017).
Pediatr Allergy Immunol. 2017 Jun;28(4):370-376. IF:3.775*
** The impakt factor of the given year is not available*

14. Csuka D, Veszeli N, Varga L, Prohászka Z, Farkas H. The role of the complement system in hereditary angioedema. (2017).
Mol Immunol. 2017 Sep;89:59-68.
The journal IF in 2016 is 3.236, but due to the classification of the publication it is not listed.

15. Kessel A, Farkas H, Kivity S, Veszeli N, Kőhalmi KV, Engel-Yeger B. Therelationship between anxiety and quality of life in children with hereditaryangioedema. (2017)
Pediatr Allergy Immunol. 2017 Jul 10. IF:3.775*
** The impakt factor of the given year is not available*

16. Kőhalmi KV, Veszeli N, Luczay A, Varga L, Farkas H. A danazolkezelés hatása C1-inhibitor-hiány okozta herediter angiooedemás gyermekek növekedésére. (2017)
Orv Hetil. 2017 Aug;158(32):1269-1276. IF:0.349*
** The impakt factor of the given year is not available*

17. Hofman ZLM, de Maat S, Suffritti C, Zanichelli A, van Doorn C, Sebastian SAE, Veszeli N, Csuka D, Renné T, Pasterkamp G, Cicardi M, Farkas H, Hack CE, Maas C. Cleaved kininogen as a biomarker for bradykinin release in hereditary angioedema. (2017)
J Allergy Clin Immunol. 2017 Aug 4. pii: S0091-6749(17)31268-X. IF:13.081*
** The impakt factor of the given year is not available*

Cumulative IF of the publications unrelated to the subject of the PhD thesis: **52.74**