

PROF. HENRIETTE FARKAS (Orcid ID : 0000-0003-2929-1721)

DR. LILIAN VARGA (Orcid ID : 0000-0002-5484-364X)

Article type : Brief Communication

Complete kinetic follow-up of symptoms and complement parameters during a hereditary angioedema attack

Short title: Complete kinetic follow-up of a hereditary angioedema attack

Nóra Veszeli MSc^{1,2}, Kinga Viktória Köhalmi MD^{1,2}, Erika Kajdácshi PhD¹, Dominik Gulyás BSc¹, György Temesszentandrás MD², László Cervenak PhD¹, Henriette Farkas MD, PhD, DSc^{1,2}, Lilian Varga PhD^{1,2}

¹Research Laboratory, 3rd Department of Internal Medicine, Semmelweis University, Hungary

²Hungarian Angioedema Center, 3rd Department of Internal Medicine, Semmelweis University, Hungary

Corresponding author:

Lilian Varga PhD

Hungarian Angioedema Center,
3rd Department of Internal Medicine,
Semmelweis University, Budapest, Hungary

H-1125 Budapest, Kútvölgyi út 4, Hungary

Telephone: (+36 1) 325-1481, Fax: (+36 1) 225 3899

E-mail: varga.lilian@med.semmelweis-univ.hu

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13327

This article is protected by copyright. All rights reserved.

Declaration on all sources of funding:

This study was supported by the grants OTKA 112110 (LV) and by the ÚNKP-16-3 New National Excellence Program of the Ministry of Human Capacities.

Conflict of interest statement: Henriette Farkas has received consultancy/speaker fees and honoraria from Shire Human Genetic Therapies Inc., Swedish Orphan Biovitrum, and CSL Behring. Other authors have no conflict of interest.

Abstract

We studied the kinetics of C1-inhibitor (C1-INH) and other complement parameters in a self-limited edematous attack (EA) in a patient with hereditary angioedema due to C1-INH deficiency to better understand the pathomechanism of the evolution, course, and complete resolution of EAs. C1-INH concentration and functional activity (C1-INH_{c+f}), C1(q,r,s), C3, C4, C3a, C4a, C5a and SC5b-9 levels were measured in blood samples obtained during the 96-hour observation period. The highest C1-INH_{c+f}, C4, and C1(q,r,s) levels were measured at baseline, and their continuous decrease was observed during the entire observation period. C4 depletion started at prodromal phase and C4 was lowest after the maximum severity peak. Compared to baseline, C4a level was four times higher 7 hours before the onset of the attack. C1-INH did not increase after resolution of the attack suggesting that factors other than C1-INH may be important in this process. C4a may be a useful biomarker for the prediction of EAs.

Key words: complement, edematous attack, hereditary angioedema with C1-inhibitor deficiency, kinetic follow-up, prodromal period

Abbreviations: **C1-INH-HAE**= Hereditary angioedema with C1-inhibitor deficiency; **C1-INH** = C1-inhibitor; **C1-INH_c**= concentration of C1-inhibitor; **C1-INH_f**= functional activity of C1-inhibitor; **CV%**=coefficient of variation; **EA**= edematous attack; **pdC1-INH**= plasma-derived C1-inhibitor concentrate; **VAS**= visual analog scale

Introduction

The pathomechanism and the biological background of the evolution, course, and complete resolution of edematous attacks (EAs) in patients with hereditary angioedema with C1-inhibitor deficiency (C1-INH-HAE) are not completely understood. Their exploration is complicated because neither the location, nor the severity of the subsequent edema formation can be predicted in advance. In the majority of patients, the onset of edema is often preceded by prodromal symptoms; however, these are not always followed by an EA. The most objective prodromal symptom of C1-INH-HAE is erythema marginatum (1-3).

Kinetic monitoring of the factors involved in the pathogenesis of C1-INH-HAE attacks would be highly important, however, so far, only pharmacokinetic and pharmacodynamic studies have been conducted in C1-INH-HAE, and these did not focus on understanding the kinetics of spontaneously resolving EAs (4-6). One of our C1-INH-HAE patients – who usually experiences EAs preceded by prodromal symptoms – has volunteered to be studied. This allowed our team to follow and document the natural course of an EA.

Our objective was to observe and describe the evolution of clinical manifestations and the changes in their severity, starting from the symptom-free state until the patient became symptom-free again. Further, we intended to determine whether a relationship exists between C1-INH levels and clinical symptoms. Finally, as C1r/s is exclusively regulated by C1-INH,

we analyzed the concentration of several key components of the classical pathway in correlation with the clinical symptoms.

Subjects & Methods

The patient and the control subject

A 56-year old female patient with C1-INH-HAE type I volunteered to be studied. The patient experienced, on average 26 EAs per year over the last two years. Ninety-five per cent of these EAs were preceded by erythema marginatum, which was always followed by an attack. The patient experienced the latest EA 9 days before the study; treatment with 1000 IU plasma-derived, C1-inhibitor concentrate (pdC1-INH, Berinert, CSL Behring) achieved complete resolution of the symptoms in 2 days. Three days before the first day of our study, she received a further dose of 1000 IU pdC1-INH as prophylaxis, in preparation for a family event. The patient has not had any other diseases and did not receive any medicinal products during the study. During the complete follow-up period the patient recorded the symptoms, using a visual analog scale (VAS; 0-100 mm scale).

The control subject was a 52-year-old, healthy female volunteer. C1-INH deficiency was excluded by complement testing.

Both subjects gave informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the Hungarian Regulatory Authority (BPR/021/09599-7/2014).

Blood sampling

In total, 12 blood samples were drawn from the patient. One was obtained at baseline, in the symptom-free state. The second sample was taken a day later, upon hospitalization; 45 minutes after prodromal symptoms had been identified by the patient. Subsequently, blood sampling was repeated at 6-hour intervals. Four samples were obtained in the prodromal

period, and another four during the EA. The patient notified us about the onset of edema, therefore we could obtain an extra sample at the beginning of the attack. The penultimate blood sample was drawn at the time of complete resolution of symptoms. The final sample was obtained also during symptom-free state, a day later.

We collected blood samples from a healthy control subject at 6-hour intervals (synchronized with the sampling performed on the patient) over a 24-hour period in order to have the possibility to taking into account the circadian rhythm of the studied parameters.

All serum and EDTA-plasma samples were stored at -80°C until processed.

The determination of complement parameters

All analyzed parameters were determined using the same unfrozen aliquot from each subject. All samples were measured in parallel.

Commercial kits were used to determine the functional activity of C1-inhibitor (C1-INH_f) in serum as well as the concentration of C3a, C4a, C5a and SC5b-9 in EDTA-plasma (Quidel, San Diego, USA) samples. Radial immunodiffusion was performed to measure C1r concentration (R&D Systems, Minneapolis, USA). C3 and C4 concentration were measured by turbidimetry (Cobas Integra 400 analyzer; Roche, Switzerland).

The concentration of C1s and C1-INH (C1-INH_c) were determined by in-house sandwich ELISA-methods using goat and mouse anti-human C1s (Quidel, San Diego, USA and Thermo Fisher Scientific, Rockford, USA) as regards C1s, and rabbit anti-human C1-INH antibodies as regards C1-INH.

Results

Clinical signs and symptoms

Nineteen hours before the onset of the EA, prodromal symptoms appeared as nausea, skin tension on the thighs and on the left upper arm, then stiffness occurred in the fingers of the left hand. The patient experienced erythema marginatum occurred at various locations. The prodromal symptoms persisted throughout the EA and resolved (VAS score ≤ 3) simultaneously with the edema (Figure 1).

Edema first occurred on the left hand. Two hours later, the swelling of the hand increased, and edema appeared on the right thigh. The severity of the edematous symptoms had peaked 5.5 to 8.5 hours after onset, and started to improve thereafter. The complete resolution of EA (VAS ≤ 3) took 30 hours from onset (Figure 1). A day later, final follow-up visit was performed, also during symptom-free state. The patient was monitored for 96 hours altogether.

Complement analysis

The high resolution kinetics of C1-INH_{C+I} as well as the levels of C1q, C4 and its activation product (C4a) along with the level of C3 during the 96-hour observation period in the patient as well as during the 24-hour observation period in the healthy control are shown in details in Figure 2.

C1r and C1s concentration (data not shown) exhibited similar kinetics to that of C1q. The coefficient of variation (CV%) values measured during the observation period were 12.1 for C1r and 19.1 for C1s. In the healthy control subject, C1r and C1s levels did not show unidirectional changes, and its fluctuation was smaller (CV%: 8.9 and 14.4 respectively) than that seen in the patient.

The concentration of C3a, C5a, and SC5b-9 complement activation products in the patient did not show increasing tendency, and their levels measured during the observation period was lower than that of the healthy control. The CV% values were 20.5 vs. 7.8 of C3a, 15.5 vs. 7.9 of C5a and 17.9 vs. 21.5 of SC5b-9 (patients vs. control).

Discussion

This is the most complete follow-up study describing the natural course of an EA in C1-INH-HAE – from the symptom-free state, until the complete resolution of EA without acute treatment. Our unique study describes the prodromal and edematous symptoms and their severity separately. This made possible ‘real-time’ exploration of the pathophysiology of the EA, by analyzing the monitored parameters together with the underlying clinical events.

Although a correlation between C1-INH levels and the clinical symptoms would appear evident (7, 8), so far, only a single study explored the possibility to predict the onset of EAs by monitoring C1-INH levels. SPATH *et al.* theoretically found that if C1-INH_c is below a critical value (around 0.035 g/L) the onset of an attack has the highest probability (9). In our patient, 0.033 g/L C1-INH_c was measured at onset of edematous symptoms, which supports the above estimation, and it seems that C1-INH level is indeed critical factor in the onset of symptoms. On the other hand, as the level of C1-INH remained negligible after the spontaneous resolution of the EA, it suggests that the spontaneous resolution of edematous symptoms may depend on other factor(s) than C1-INH.

Earlier studies suggested that the classical pathway is activated during EAs in C1-INH-HAE (10, 11). We showed for the first time that C4 was activated at the onset of prodromal symptoms and its level decreased continuously until depletion, whereas the level of C4a changed inversely. It suggests that C4a could become a useful biomarker for predicting the occurrence of EAs. In our knowledge, such a biomarker is not currently available (12).

We can suppose a correlation between the course of edema formation, and the changes in C3 level, because in the healthy control subject, C3 levels did not change much over 24 hours, so these changes could not have happened because of the circadian rhythm of C3. We found that the decrease of C3 level during the EA was not accompanied by the elevation of C3a, C5a and SC5b-9 levels. As the latter were always lower in the patient than in the healthy control subject, it appears that the activation of the classical complement pathway is down-regulated at the level of C3, and additional complement activation cannot be detected. This finding is partly supported by literature data on the comparison of blood samples obtained in symptom-free periods, or during EAs, however Nielsen et al. have found moderately elevated SC5b-9 levels during EAs (11, 13).

Although every EA and each patient might have different characteristics, our findings may provide a solid basis for further kinetics research.

Our findings indicate concomitant impairment of the regulation of further plasma enzyme systems controlled by C1-INH (kinin-kallikrein, coagulation and fibrinolytic systems) (14). Therefore we plan to follow-up also these systems, in particular those parameters which may have potential role in increased vasopermeability and subsequently in the development of EAs.

Author's contributions: **NV:** Contribution to concept and design of the study, acquisition, analysis and interpretation of data, drafting/writing the article and revising it, final approval of the version to be published, **KVK:** Contribution to concept and design of the study, acquisition of data, drafting the article, final approval of the version to be published, **EK:** Contribution to concept and design of the study, acquisition and analysis of data, revising the article critically, final approval of the version to be published, **DG:** acquisition of data, drafting the article, final approval of the version to be published, **GYT:** acquisition of data, revising the article, final approval of the version to be published, **LC:** Contribution to concept and design of the study analysis and interpretation of data, revising the article critically, final approval of the version to be published, **HF:** Contribution to concept and design of the study, interpretation of data, revising the article critically, final approval of the version to be published, **LV:** Contribution to concept and design of the study, interpretation of data, drafting the article and revising it critically, final approval of the version to be published.

References

1. Magerl M, Doumoulakis G, Kalkounou I, Weller K, Church MK, Kreuz W, et al. Characterization of prodromal symptoms in a large population of patients with hereditary angio-oedema. Clin Exp Dermatol. 2014;39(3):298-303.
2. Reshef A, Prematta MJ, Craig TJ. Signs and symptoms preceding acute attacks of hereditary angioedema: results of three recent surveys. Allergy Asthma Proc. 2013;34(3):261-6.
3. Farkas H, Harmat G, Fay A, Fekete B, Karadi I, Visy B, et al. Erythema marginatum preceding an acute oedematous attack of hereditary angioneurotic oedema. Acta Derm Venereol. 2001;81(5):376-7.

4. Kusuma A, Relan A, Knulst AC, Moldovan D, Zuraw B, Cicardi M, et al. Clinical impact of peripheral attacks in hereditary angioedema patients. *Am J Med.* 2012;125(9):937 e17-24.
5. Farrell C, Hayes S, Relan A, van Amersfoort ES, Pijpstra R, Hack CE. Population pharmacokinetics of recombinant human C1 inhibitor in patients with hereditary angioedema. *Br J Clin Pharmacol.* 2013;76(6):897-907.
6. van Doorn MB, Burggraaf J, van Dam T, Eerenberg A, Levi M, Hack CE, et al. A phase I study of recombinant human C1 inhibitor in asymptomatic patients with hereditary angioedema. *J Allergy Clin Immunol.* 2005;116(4):876-83.
7. Kelemen Z, Moldovan D, Mihaly E, Visy B, Szeplaki G, Csuka D, et al. Baseline level of functional C1-inhibitor correlates with disease severity scores in hereditary angioedema. *Clin Immunol.* 2010;134(3):354-8.
8. Csuka D, Fust G, Farkas H, Varga L. Parameters of the classical complement pathway predict disease severity in hereditary angioedema. *Clin Immunol.* 2011;139(1):85-93.
9. Spath PJ, Wuthrich B, Butler R. Quantification of C1-inhibitor functional activities by immunodiffusion assay in plasma of patients with hereditary angioedema--evidence of a functionally critical level of C1-inhibitor concentration. *Complement.* 1984;1(3):147-59.
10. Cugno M, Nuijens J, Hack E, Eerenberg A, Frangi D, Agostoni A, et al. Plasma levels of C1- inhibitor complexes and cleaved C1- inhibitor in patients with hereditary angioneurotic edema. *J Clin Invest.* 1990;85(4):1215-20.
11. Nielsen EW, Johansen HT, Hogasen K, Wullemmin W, Hack CE, Mollnes TE. Activation of the complement, coagulation, fibrinolytic and kallikrein-kinin systems during attacks of hereditary angioedema. *Scand J Immunol.* 1996;44(2):185-92.
12. Farkas H, Veszeli N, Kajdacs E, Cervenak L, Varga L. "Nuts and Bolts" of Laboratory Evaluation of Angioedema. *Clin Rev Allergy Immunol.* 2016;51(2):140-51.

13. Veszeli N, Csuka D, Zotter Z, Imreh E, Jozsi M, Benedek S, et al. Neutrophil activation during attacks in patients with hereditary angioedema due to C1-inhibitor deficiency. *Orphanet J Rare Dis.* 2015;10(1):156.

14. Csuka D, Veszeli N, Imreh E, Zotter Z, Skopal J, Prohaszka Z, et al. 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;10:132.

Figure legends

Figure 1. Follow-up of the severity of prodromal (open circles) and edematous symptoms (closed circles) during the 96-hour observation period in a C1-INH-HAE patient. White background refer to 'during symptom-free period', light grey background refer to 'during prodromal period', dark grey background refer to 'during edematous attack' of the patient.

Figure 2. Kinetic follow-up of C1-INH concentration (C1-INH_c) (A), C1-INH functional activity (C1-INH_f) (B), C1q (C), C4 (D), C4a (E) and C3 (F) in the patient (closed circles) during 96-hour observation period and in the healthy control (open circles) during 24-hour period. In the patient C1-INH_{c+f} (A, B) were the highest at baseline, when the patient was still symptom-free and both indices continued to decrease throughout the observation period. C1-INH_{c+f} did not increase during the improvement, or after the complete resolution of symptoms. C1q concentration (C) decreased compared to baseline and did not return to baseline even after the complete resolution of symptoms. C4 concentration (D) was the highest at baseline, it started to decrease upon the onset of prodromal symptoms, and its decline increased during the EA. The trend of the decline did not change even during the improvement of symptoms. C4 level had decreased below the detection threshold, and did not improve during the remainder of the study period. The concentration of the C4a activation product (E) began to increase as early as during the prodromal period. In the seventh hour

Accepted Article

before the onset of the attack, its value was four times higher than at baseline. C4a level stayed high during the edematous attack, it began to decrease during the improvement, and returned to baseline upon the resolution of symptoms. C3 level (F) began to fluctuate at the onset of prodromal symptoms. Then, during the edematous attack, it declined below the reference range parallel with the improvement of symptoms. In the healthy control subject, all parameters were stable throughout the observation period. Dotted lines represent the reference ranges which are 0.15 to 0.3 g/l for C1-INH concentration, 70 to 110 % for C1-INH functional activity, 60-180 mg/l for C1q, 0.15-0.55 g/l for C4 and 0.9-1.8 g/l for C3, based on our diagnostic laboratory measurement. For C4a, our laboratory did not have reference range because we did not measure this parameter in the routine diagnostics. White background refer to 'during symptom-free period', light grey background refer to 'during prodromal period', dark grey background refer to 'during edematous attack' of the patient.

