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Bradykinin and oxidative stress in patients with hereditary angioedema due to C1 inhibitor deficiency

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Short title: Bradykinin and oxidative stress in hereditary angioedema

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Conflict of interest: none declared.
C1 inhibitor deficiency (C1-INH-HAE) is a rare heredity disease in which deficiency of C1 inhibitor (C1-INH) disturbs homeostasis balance of many systems, metabolism and immunity. We have examined: the level of indicators of redox balance (the level of basal and H2O2 induced reactive oxygen species - ROS as well as plasma advanced glycation end products - AGEs and advanced oxidation protein products - AOPPs) in patients with C1-INH-HAE type I/II, and the influence of exogenous bradykinin on ROS level.

The results of our study are the first, which revealed significant increase of basal and H2O2 induced ROS in patients with C1-INH-HAE as well as an antioxidant action of exogenous bradykinin on these both phenomena.
ABSTRACT

Introduction: Hereditary angioedema (HAE) is a rare autosomal dominant disease caused by genetic dysfunction of C1 inhibitor (C1-INH) due to mutations in the SERPING1 gene. The disorder is mediated mainly by bradykinin. The clinical course of the disease is varied and not related to genetic changes.

Objectives: We aimed to evaluate redox homeostasis of peripheral blood mononuclear cells (PBMCs) in patients with HAE due to C1-INH deficiency (C1-INH-HAE) by measuring the levels of reactive oxygen species (ROS) of peripheral blood mononuclear cells (PBMCs) as well as plasma advanced glycation end products (AGEs) and advanced oxidation protein products (AOPPs). We also aimed to assess the effect of bradykinin on ROS levels.

Patients and methods: We enrolled 30 adults with C1-INH-HAE and 15 healthy individuals. The levels of ROS were measured by flow cytometry, while the plasma levels of AGEs and AOPPs were determined spectrophotometrically by enzyme-linked immunosorbent assays.

Results: Basal and hydrogen peroxide (H$_2$O$_2$)-induced ROS levels were higher in patients with HAE when compared with controls ($P = 0.002$ and $P = 0.001$, respectively), indicating abnormalities in redox homeostasis. Plasma AOPP and AGE levels were also higher, but the differences were not significant. Bradykinin significantly reduced basal and H$_2$O$_2$-induced ROS generation in PBMCs only in patients with HAE ($P = 0.03$).

Conclusions: The higher basal and H$_2$O$_2$-induced ROS levels in patients with C1-INH-HAE indicate the redox imbalance. However, by reducing basal and H$_2$O$_2$-induced ROS levels, bradykinin shows antioxidant action in this disorder.

Key words: antioxidant, bradykinin, C1 inhibitor, hereditary angioedema, oxidative stress
INTRODUCTION

Hereditary angioedema (HAE) due to C1 inhibitor (C1-INH) deficiency (C1-INH-HAE) is a rare autosomal dominant disease caused by C1-INH deficiency (type I – 85% of patients with low antigenic and functional plasma level of C1-INH) or C1-INH dysfunction (type II 15% of patients) with normal or elevated plasma level of C1-INH and low functional level of C1-INH [1]. C1-INH is essential for numerous physiological processes, including complement and coagulation systems as well as fibrinolysis and kinin system [1-5]. The disease is caused by heterogeneous mutations within the C1-INH gene (SERPING1) [6-7]. Despite a considerable progress in the understanding of the pathological mechanism of this disorder, a number of phenomena have not been elucidated.

One area of the ongoing open question is lack of the correlation between phenotype and genotype of patients with HAE [1,6,7]. Also, despite constantly low level and activity of C1-INH some patients periodically are asymptomatic or suffer from of angioedema [8]. Another interesting aspect is related to the course of the disease, which is different among the persons from the same family despite similar genetic changes in C1-INH gene [9]. As indicated by previous studies, neither the type of genetic changes nor complement parameters allow for the prognosis of the clinical course of the disease [2,7,9].

It is generally accepted that bradykinin is the main mediator inducing an angioedema attack in patients with C1-INH-HAE [2-5,10-12]. Bradykinin is a tissue hormone generated by the kinin system as an inflammatory product of the coagulation system [1,2,3,10-12]. It acts via bradykinin B2 receptor and is very quickly inactivated. However, its metabolites act via actively formed bradykinin B1 receptors. Bradykinin exerts several physiological actions. It increases the permeability of capillaries, leading to local edema, warming, and erythema. Its vasodilating activity results in the release of three potent mediators: tissue plasminogen activator, prostaeyclin, and endothelium-derived vascular relaxing factor. Bradykinin-
mediated angioedema seems to occur in individuals with hereditary or acquired C1-INH deficiency owing to an easy activation of the complement system, plasma contact system, and kinin system as a result of uncontrolled overproduction of kallikrein and in consequence, of bradykinin. There may be various direct reasons for the increase of serum bradykinin levels inducing angioedema in a given patient, which are often difficult to establish.

Patients with C1-INH-HAE report diverse unspecific triggers to provoke attacks of edema such as trauma or injury, pressure, effort, stress, or infection [1-3,13]. Bradykinin-mediated angioedema may be also caused by various metabolic products and environmental agents [8]. These diversity of triggers in patients with C1-INH-HAE suggests the presence of reactions based on a feedback phenomenon, allowing the human body to adapt to the changing internal or external environment independently from the stimulus, as well as to maintain homeostasis, in which-appropriate redox balance may play an essential role.

The aim of the study was to evaluate oxidative stress in patients with C1-INH deficiency by measuring basal and hydrogen peroxide (H$_2$O$_2$)-induced levels of ROS in peripheral blood mononuclear cells (PBMCs) as well as by measuring the plasma concentrations of advanced glycation end products (AGEs) and advanced oxidation protein products (AOPPs). Moreover, we evaluated the effect of bradykinin on basal and H$_2$O$_2$-induced levels of ROS in vitro.

**PATIENTS AND METHODS**

The study included 30 patients (21 women and 9 men) with C1-INH-HAE type I and II, diagnosed and remaining under the care of the HAE Center in Krakow (Table 1).

The diagnosis of C1-INH-HAE was established on the basis of the patient and family history, on the examination during attacks of angioedema and reduced C1-INH antigen serum level as well as C1-INH activity below 50% of the reference values according to international
Measurement of intracellular reactive oxygen species generation

Anticoagulant-treated blood was collected in EDTA tubes and layered over lymphocyte separation medium (Ficoll, Pan-Biotech GmbH, Aidenbach, Germany), enabling PMBC isolation by centrifugation in a density gradient medium (400 g, 30 minutes, 20°C).

The control group included 15 healthy individuals (11 women and 4 men; mean age, 48 years; range, 30-61 years). All controls had a negative family history of HAE and normal serum levels of C1-INH (median range, 0.21-0.39 g/l; reference range, 0.21-0.39 g/l), functional activity of C1-INH (median range, 96.6% [74%-119%]; reference range, 70%), and serum C4 levels (median range, 0.21-0.39 g/l; reference range, 0.1-0.4 g/l).

The Ethics Committee of Jagiellonian University in Krakow approved the study (104/B2014, 22 May 2014) and patients signed a written informed consent. The study was carried out in accordance with the Declaration of Helsinki and its amendments.

The biochemical diagnosis of C1-INH-HAE was performed during remission of symptoms and was based on the measurement of serum C1-INH and complement component C4 levels as well as functional activity of plasma C1-INH. Serum C1-INH and C4 levels were measured by nephelometry with specific C1-INH antiserum (Siemens Healthcare Diagnostics GmbH, Marburg, Germany), and the functional level of C1-INH was determined using the functional chromogenic assay Berichrom C1-INH (Siemens Healthcare Diagnostics GmbH).
United Kingdom) supplemented with 1% fetal bovine serum (Gibco) (400 g, 6 minutes, 4°C), and then counted using the Bürker chamber.

To detect ROS generation, flow cytometry with 2’,7’-dichlorodihydrofluorescein diacetate (DCFH-DA) dye (Sigma-Aldrich, St. Louis, MO, USA) was used, according to Wang et al. [15] and Sarkar et al. [16]. DCFH-DA is a stable nonfluorescent and cell-permeable compound, which is converted within the cell to nonfluorescent DCFH by intracellular esterases. The deesterified product is oxidized by intracellular ROS to the highly fluorescent 2’7’-dichlorofluorescein (DCF). The intensity of green fluorescence upon excitation at 488 nm is proportional to the intracellular level of ROS. For this assay, PBMCs isolated from blood were cultured in medium for 24 hours. Then, the cells were washed with Krebs–Ringer–Hepes (KRH) buffer, transferred into 2 tubes, and incubated with or without 100-pM bradykinin in KRH buffer for 10 minutes. After that, both samples were loaded with 100-µM DCFH-DA in KRH buffer. After incubation in 5% CO₂/95% air at 37°C for 45 minutes in dark, the cells were washed, divided into 2 aliquots, and suspended in KRH buffer. The free radical generator H₂O₂ (1 µM) was added extracellularly to one sample for 10 minutes. Then, basal and H₂O₂-induced ROS levels were measured in PBMCs. DCF fluorescence was measured by flow cytometry (FACS Canto II, Becton Dickinson, San Jose, California, United States). Data were acquired and analyzed using the DIVA software (Becton Dickinson).

DCFH-DA was dissolved in dimethyl sulfoxide (Sigma) as the stock solution and kept frozen at a temperature of -20°C. For cell loading, DCFH-DA from stock solution was mixed with KRH loading buffer (NaCl, 116 mM; KCl, 4 mM; MgCl₂, 1 mM; CaCl₂, 1.8 mM; glucose, 25 mM; HEPES acid, 10 mM; pH adjusted to 7.4) to a final concentration of 100 µM. Bradykinin (Bachem, Bubendorf, Switzerland) was dissolved in water as the stock solution and kept frozen at a temperature of -20°C until mixed with the loading buffer to a
final concentration of 100 pM. H$_2$O$_2$ (Farmina, Kraków, Poland) was diluted in the loading buffer to a final concentration of 1 mM.

The plasma concentrations of AGEs and AOPPs were measured spectrophotometrically using commercially available enzyme-linked immunosorbent assay kits (Bioassay Technology Laboratory, Shanghai, China) and a microplate reader (Multiskan, Thermo Fisher Scientific, Waltham, Massachusetts, United States). Each sample was analyzed in duplicate.

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of variable distribution, and the Levene test was used to assess the homogeneity of variance. Data were presented as median and lower and upper quartiles [Q1; Q3]. Box and whisker plots were generated with Statistics v13.0 (Statsoft Inc., Tulsa, Oklahoma, United States) and show median, interquartile range [IQR], and minimum and maximum values. The Mann-Whitney test was used to compare patient and control groups as well as to compare bradykinin-treated and -untreated PBMCs. The significance level was set at a $P$ value of less than 0.05. Spearman’s Rank correlation test was used to find the correlations between AOPP and AGE’s values both in the study and the reference groups. Analyses were carried out, using the Statistica software package version 13.0 (Statsoft Inc., Tulsa, USA).

RESULTS

Basal ROS levels, expressed as DCF fluorescence intensity in relative fluorescence units (RFUs), were higher in PBMCs isolated from patients with HAE than in those obtained from controls (median [Q1; Q3], 2967 [2634; 3800] RFUs vs 2147 [1690; 2786] RFUs; $P = 0.002$) (Figure 1). H$_2$O$_2$-induced ROS levels were also higher in PBMCs obtained from HAE patients than in those from healthy controls (median [Q1; Q3]: 6672 [5140; 10494] RFUs vs
4352 [3390; 5531] RFUs; \( P = 0.001 \), indicating that patients with HAE have disturbances in redox homeostasis (Figure 2).

Median plasma levels of AOPPs and AGEs were no significantly higher in the patient group than in controls (\( P = 0.28 \) and \( P = 0.5 \), respectively) (Figures 3 and 4). Increased AOPP levels in patients with HAE were positively correlated with increased AGE values (\( r = 0.93, P < 0.00001 \)). A similar correlation was observed in controls (\( r = 0.93, P < 0.00001 \)).

In our study, exogenous bradykinin was shown to act as an antioxidant by lowering basal and \( \text{H}_2\text{O}_2 \)-induced ROS levels in PBMCs. Exposure to bradykinin reduced basal ROS levels in patients with HAE (median [Q1; Q3], 2967 [634; 3800] vs 2617 [2119; 3051]; \( P = 0.03 \)) (Figure 5A), but not in controls (median [Q1; Q3], 2147 [1690; 2786] vs 2022 [1347; 2392]; \( P = 0.31 \)) (Figure 5B). Moreover, exposure to bradykinin decreased also \( \text{H}_2\text{O}_2 \)-induced ROS levels in patients with HAE (median [IQR], 6672 [5140-10494] vs 5554 [3854-6989]; \( P = 0.046 \)) (Figure 6A), but not in controls, in whom no effect of bradykinin was observed (median [IQR], 4352 [3390-5531] vs 4126 [2726-4486]; \( P = 0.23 \)) (Figure 6B).

**DISCUSSION**

The results of our study show as the first that patients with type I or II C1-INH-HAE had increased levels of basal ROS in PBMC as compared with controls, which confirms that they have impaired redox balance with significant prevalence of free radicals.

Moreover, we found that the use of \( \text{H}_2\text{O}_2 \) as a nonspecific stimulus applied to PBMCs significantly increased ROS levels in patients with C1-INH-HAE when compared with controls. This indicates an increased tendency of PBMCs to produce free radicals when subjected to nonspecific stimuli and confirm disturbances in the redox homeostasis in this patient group.
In our study, we used DCFH-DA to monitor changes in the redox status in PBMCs in response to the activation with an oxidative stimulus, because DCFH can be oxidized by various ROS. Thus, an increase of intracellular DCF fluorescence reflects the overall oxidative stress index in cells. Oxidative stress occurs in cells when the generation of ROS overwhelms the cells’ natural antioxidant defenses. DCFH-DA may be used as a redox indicator probe that responds to changes in intracellular iron signaling or peroxynitrite formation [17].

Moreover in the study we used advanced oxidation protein products (AOPPs) and advanced glycation end products (AGEs) modified by interactions with ROS in the blood as an additional biomarkers of oxidative stress tend to be elevated in C1-INH-HAE patients, also the difference did not reach statistical significance. We observed that AGEs values >1000 ng/l were presented only in plasma samples from C1-INH-HAE not healthy controls. In the paper by del Giacco et al. [18], both C1-INH-HAE and FXII HAE groups were examined and the results showed that AGE levels were elevated only in the C1-INH-HAE group not in the FXII-HAE group, compared to control. Contrary, C1-INH deficiency did not seem to affect AOPPs levels in cited study, as both C1-INH-HAE and the FXII-HAE groups showed elevated AOPPs levels, compared to controls. Therefore, further studies on a larger group of patients and using the same measurement methods are needed to verify the values of these promising and very important parameters in studies concerning the oxidative stress involved in HAE pathophysiology.

Oxidative stress results from an imbalance between endogenous production of free ROS and inadequate effectiveness of antioxidant defense mechanisms. This imbalance can worsen inflammation and injury conditions by enhancing the release of proinflammatory cytokines and altering enzymatic function [10,19], by activating the complement [20-22] and kinin system as well as kinin receptors [23-27], by endothelial cell activation and
dysfunction [25,28-31], as well as by affecting gene expression [32]. C1 inhibitor deficiency can aggravate these effects, especially by impaired redox homeostasis and increased oxidative stress in patients with C1-INH-HAE, as shown by our study and by Del Giacco et al. [18].

The study results concerning the influence of exogenous bradykinin on basal and H$_2$O$_2$-induced ROS levels in PBMCs in patients with C1-INH-HAE showed that bradykinin had a significant antioxidant effect in this group of patients. Lack of this bradykinin action in health control may be associated with normal C1-INH level or may indicate that antioxidant effect of bradykinin is revealed only in case of increased oxidative stress.

To our knowledge, this is the first study confirming the antioxidant action of bradykinin in C1-INH-HAE. This seems to corroborate the findings reported so far in animal models [33,34] as well as experimental studies on degenerative disease and cell aging processes in humans [24,25,35-39].

The study results concerning as well an oxidative stress in C1-INH-HAE as an influence of bradykinin on oxidative stress of PBMC in patients with C1-INH-HAE were presented as preliminary findings during Bradykinin Symposium 2018 in Berlin [40]. They are generally consistent with a recent study by Del Giacco et al. [18] indicating on the role of oxidative stress in pathophysiology of C1-INH-HAE.

We focus on bradykinin effect on PBMC, as cells separated from easily accessible specimen, thus we chose flow cytometry analysis using dihydrochlorofluorescein diacetate (DCFH-DA) as fluorescent probe. Flow cytometry is one of the most powerful tools for single-cell analysis of the immune system used for many years to evaluate oxidative burst in many conditions, such as autoimmune neutropenia and asymptomatic HIV + individuals [41]. DCFH-DA have widely been used for ROS/RNS detection, especially in mononuclear leukocytes (whereas other fluorescent probes are used only for polymorphonuclear
leukocytes). This probe detects more reactive species (HO●, ONOO-, ROO●, NO2●, H2O2) than other probes. Isolation of PBMC from whole blood and stabilization in the medium allowed to avoid some factors interfering with ROS determination [42].

Although the major effects of bradykinin are exerted on endothelium, previous data showed the expression of B2R and B1R on the surface of PBMC, indicating another target cells for bradykinin action [43]. Although B1R is a potent activator of inducible nitric oxide and NADPH oxidase, potentially leading to oxidative stress, the role of B2R in this process is unclear. Based on the recent study on the role of bradykinin in neuroprotection, the molecular mechanisms of action of bradykinin involve down-regulation of the caspase-1, IL-1β and IL-18 levels and Cleaved GSDMD (a key executioner of pyroptosis) expression [44]. Moreover, human tissue kallikrein gene delivery was found to protect against cerebral ischemia/reperfusion injury through bradykinin B2 receptor activation and the propose novel signaling mechanisms involved activation of Homer1b/c-ERK1/2 and Homer1b/c-PI3K-Akt signaling pathways [45]. Based on our study, the next step in further research on antioxidant activity of bradykinin in the model of on PBMCs in patients with C1-INH-HAE, should be transcriptomics analysis of the signaling pathways.

Patients with C1-INH-HAE have significantly elevated intracellular ROS level in PBMC compared to healthy subjects. In case of adverse process which is oxidative stress, when redox balance is disturbed, different antioxidants may decrease ROS level to some extent. Nevertheless, some amount of free radicals in healthy controls’ cells are needed as signaling molecules and ROS are generated continuously in physiological processes thus this so called ‘oxidative eustress’ is not affected by antioxidants. It is known, that cytokines produced by macrophages and mast cells, occurring in various diseases including HAE, promote overexpression of NADPH oxidase, which is the main source of excess ROS (besides nitric oxide synthase and xanthine oxidase) in ischemia/reperfusion injury [18,23,34].
Recently, it has been reported, that in the state of hypoxia/reperfusion injury – mediated oxidative stress, the expression of B2R significantly increase [44,45]. Bradykinin B2 receptors may play a neuroprotective role in hypoxia/reoxygenation injury related to pyroptosis pathway. It could be hypothesized that the same up-regulation of B2R expression take place in state of oxidative stress documented in C1-INH-HAE patients. However, full explanation of the role B1/B2 bradykinin receptors as well as endothelium and oxidative stress in pathophysiology of C1-INH-HAE needs further research.

Although some previous studies have indicated the antioxidant property of bradykinin in animal models, the involvement of bradykinin in intracellular redox processes has not been fully elucidated. Bradykinin was found to protect endothelial cells against oxidative stress or enhanced ROS generation [29,31]. It seems that the effect of bradykinin on the intracellular redox state depends on the type of the receptor with which the kinins or their derivatives interact [1,18,34].

A study using bradykinin receptor-null mice indicated that nonselective stimulation of B1 and B2 receptors is likely to suppress oxidative stress and diabetic nephropathy [23], while other studies suggested that the selective activation of B2 receptor [25] and inhibition of B1 receptor could be beneficial [27,46]. Data from studies on the vascular system of diabetic rats showed that B1 receptor enhances superoxide anion radical formation by activating NADPH oxidase [18,27,46]. Moreover, it has been shown that oxidative stress caused by elevated superoxide anion production induces expression of the kinin B1 receptor in the liver and brain of diabetic rats [46-48].

In light of recent findings, the cooperation between G protein-coupled receptors makes the signal transduction pathways of active peptides even more complex [18,49]. Thus, oligomerization of bradykinin receptors and other receptors may modulate physiological processes and partially account for the discrepancies between bradykinin effects reported in
different studies. Therefore, the expression and activation of B₁ and B₂ receptors in patients with C1-INH-HAE should be investigated in further studies.

Bradykinin is known to induce nitric oxide synthase and release, which may lead to an increased production of peroxynitrite radicals, especially when antioxidant systems are not efficient. At the same time, bradykinin was proved to stimulate the activity of the antioxidant enzymes superoxide dismutase and catalase in endothelial cells [18,49]. This recent evidence corroborates a previous study reporting an increase in superoxide dismutase, catalase, and glutathione peroxidase activity in hyperglycemic rats [47,48]. On the basis of these findings as well as our data, a hypothesis may be proposed that one of the various bradykinin effects is to maintain the redox balance.

One of the limitation of our study is the usage of single concentration of bradykinin. The use of different concentrations of bradykinin on PBMC samples would be valuable, but we could not do it because of the limited amount of PBMC, which was due to the amount of blood drawn from patients in accordance with ethical standards. Thus, the concentration of bradykinin (100-pM) was chosen based on data on serum bradykinin concentration during acute attacks of angioedema, provided in reliable literature [50]. Presented study results demand the confirmation in a larger study together with the examining the dose dependency of bradykinin antioxidant action in C1-INH-HAE [34].

Moreover, exposure to bradykinin decreased also H2O2-induced ROS levels in patients with HAE (median [Q1; Q3], 6672 [5140; 10494] vs 5554 [3854; 6989]; P = 0.046) (Figure 6A), but not in controls, in whom no effect of bradykinin was observed (median [Q1; Q3], 4352 [3390; 5531] vs 4126 [2726; 4486]; P = 0.23) (Figure 6B).

It seems that in patients with C1-INH-HAE, oxidative stress disturbing redox balance may induce an increase in bradykinin levels in a feedback reaction in order to recover impaired redox homeostasis. Future research should focus on elucidating the role of kinins
and kinin receptors as well as the endothelium in the pathology of bradykinin-mediated angioedema. Further studies on the role of oxidative stress and antioxidant action of bradykinin might have an important implications for the treatment of patients with C1-INH-HAE and prevention [18,35,40].

In conclusion, the results of our study are first which revealed significant increase of basal and H202 induced ROS in patients with C1-INH-HAE as well as an antioxidant action of exogenous bradykinin on these both phenomena. It seems also that in patients with C1-INH-HAE, an increase of oxidative stress may induce an increase of bradykinin level as natural antioxidant in a way of feedback reaction provoking angioedema attack.

Further studies on the role of oxidative stress and antioxidant action of bradykinin might have an important implications for the treatment of patients with C1-INH-HAE and prevention.

CONTRIBUTION STATEMENT

KO and ECz contributed to study concept and design. AB, DM, WD, MZ, JG, and AG performed laboratory tests. KO and ECz performed medical procedures. WD and AO contributed to data collection. KO, WD, ECz, JG, AB, and BS contributed to data analysis and interpretation. KO, AB, JG, DM, and ECz contributed to manuscript drafting and revision. All authors read and approved the final manuscript.
ACKNOWLEDGMENTS

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Table 1. Clinical characteristics of patients (n=30) with type I and II C1-INH-HAE

<table>
<thead>
<tr>
<th>Sex</th>
<th>Family history 0-1</th>
<th>HAE type I, II</th>
<th>Age (n=30)</th>
<th>C1-INH reference range 0.21-0.39 g/l</th>
<th>fC1-INH reference range 70%-130%</th>
<th>C4 reference range 0.1-0.4 g/l</th>
<th>Symptom's score 0-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 W</td>
<td>1 - 15</td>
<td>I - 24</td>
<td>37.5* [26; 53]</td>
<td>type I: 0.06* [0.04; 0.069]</td>
<td>22.3* [10.30; 26.00]</td>
<td>0.049* [0.034; 0.067]</td>
<td>2* [1.25; 3.00]</td>
</tr>
<tr>
<td>9 M</td>
<td>0 - 15</td>
<td>II - 6</td>
<td>9* [26; 53]</td>
<td>type II: 0.425* [0.22; 0.683]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: C1-INH - C1 inhibitor; C4 - complement component 4; C1-INH - functional activity of C1 inhibitor; HAE - hereditary angioedema; M - man; W - woman; median* [Q1; Q3]
Figure 1. Basal reactive oxygen species (ROS) levels in peripheral blood mononuclear cells from patients with hereditary angioedema (HAE) and healthy controls

* $P < 0.05$ HAE vs control (Mann-Whitney test)
Figure 2. H$_2$O$_2$-induced reactive oxygen species (ROS) levels in peripheral blood mononuclear cells from patients with hereditary angioedema (HAE) and healthy controls

* $P <0.05$ HAE vs control (Mann-Whitney test)
Figure 3. Plasma levels of advanced oxidation protein products (AOPPs) in patients with hereditary angioedema (HAE) and healthy controls.
Figure 4. Plasma levels of advanced glycation end products (AGEs) in patients with hereditary angioedema (HAE) and healthy controls
Figure 5. Effect of bradykinin on basal reactive oxygen species levels in peripheral blood mononuclear cells (PBMCs) from patients with hereditary angioedema (A) and healthy controls (B)

* $P <0.05$ bradykinin-treated vs intact PBMCs (Mann-Whitney test)

Figure 6. Effect of bradykinin on $H_2O_2$-induced reactive oxygen species levels in peripheral blood mononuclear cells (PBMCs) from patients with hereditary angioedema (A) and healthy controls (B)

* $P <0.05$ bradykinin-treated vs intact PBMCs (Mann-Whitney test)
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