Nailfold Videocapillaroscopic Findings in Bradykinin-Mediated Angioedema

Running title: Capillaroscopy in angioedema patients

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Abstract

**Background:** Hereditary angioedema with C1-inhibitor deficiency (C1-INH-HAE) and acquired angioedema related to ACE inhibitors (ACEI-AAE) are types of bradykinin-mediated angioedema without wheals characterized by recurrent swelling episodes. Recent evidence suggests that a state of “vascular preconditioning” predisposes individuals to attacks, but no data are available on the possible structural alterations of the vessels.

**Objective:** This study aims at evaluating the features of the nailfold capillaries to highlight possible structural anomalies in patients affected by C1-INH-HAE in comparison with the healthy population and ACEI-AAE patients in comparison with hypertensive controls.

**Methods:** With nailfold videocapillaroscopy (NVC), we assessed: apical, internal, and external diameter, loop length, intercapillary distance, capillary density, distribution, and morphology. Plasma levels of vascular endothelial growth factor (VEGF)-A, VEGF-C, angiopoietin (Ang)1, and Ang2 were also measured.

**Results:** C1-INH-HAE patients (n = 34) had significant structural alterations of the capillaries compared to healthy controls (n = 28): greater intercapillary distance (216 vs 190 µm), increased apical, internal, and external diameter (28 vs 22 µm; 22 vs 20 µm; and 81 vs 65 µm, respectively), decreased density (4 vs 5 capillaries/mm²), more irregular capillary distribution, and more tortuous morphology. Apical diameter was enlarged in patients with ≥12 attacks/year. In ACEI-AAE patients, NVC showed no alterations versus hypertensive controls. NVCs performed in two C1-INH-HAE patients during attacks showed no changes compared to the remission phase.

**Conclusions:** C1-INH-HAE patients have important structural capillary alterations, confirming the involvement of microcirculation in the pathogenesis of angioedema.

**Keywords:** C1-inhibitor; hereditary angioedema; ACE-inhibitor angioedema; vascular preconditioning; capillaries
Resumen

Antecedentes: Tanto el angioedema hereditario con deficiencia de inhibidor del C1 (C1-INH-HAE) como el angioedema adquirido relacionado con los inhibidores de la ECA (ACEI-AAE), son dos tipos de angioedema mediados por bradicinina que cursan con episodios de inflamación recurrente sin acompañarse de habones. Existe evidencia de la existencia de un estado de "pre acondicionamiento vascular" que predispone a estos pacientes a los ataques, pero no hay datos disponibles sobre las posibles alteraciones estructurales de los vasos.

Objetivo: Este estudio tiene como objetivo el evaluar las características de los capilares de la base ungueal para identificar posibles anomalías estructurales en los pacientes afectados por C1-INH-HAE en comparación con la población sana, y en los pacientes con ACEI-AAE en comparación con controles con hipertensión arterial.

Métodos: Mediante video-capilaroscopia de la base ungueal (NVC), se evaluaron: los diámetros apical, interno y externo, la longitud del asa, la distancia intercapilar, la densidad capilar, su distribución y su morfología. También se midieron los niveles plasmáticos del factor de crecimiento endotelial vascular (VEGF)-A, VEGF-C, angiopoyetina (Ang)1 y Ang2.

Resultados: En los pacientes con C1-INH-HAE (n = 34) se observaron alteraciones estructurales de los capilares significativas, en comparación con los controles sanos (n = 28): mayor distancia intercapilar (216 frente a 190 µm), aumento del diámetro apical, interno y externo (28 frente a 22 µm; 22 frente a 20 µm; y 81 frente a 65 µm, respectivamente), disminución de la densidad (4 frente a 5 capilares/mm2), distribución capilar más irregular y una morfología más tortuosa. El diámetro apical fue mayor en aquellos pacientes con ≥12 ataques/año. En los pacientes con ACEI-AAE, las NVC no mostraron alteraciones al ser comparadas con las de los controles hipertensos. Las NVC realizadas en dos pacientes con C1-INH-HAE durante los ataques tampoco mostraron cambios en comparación las realizadas en la fase de remisión.

Conclusiones: Los pacientes con C1-INH-HAE tienen importantes alteraciones estructurales capilares, lo que confirma la participación de la microcirculación en la patogénesis del angioedema.

Palabras clave: inhibidor del C1; angioedema hereditario; angioedema inducido por IECA; pre acondicionamiento vascular; capilares
Introduction

Angioedema is an acute swelling of the deeper layers of the skin or mucosa resulting from a transient increase in vascular permeability induced by vasoactive mediators, such as histamine and bradykinin (BK) [1].

Histamine is the principal mediator of the mast-cell-mediated angioedema, which is characterized by the presence of wheals and itching. Conversely, angioedema mediated by BK (BK-AE) results from a variety of circumstances, such as the uncontrolled generation of BK, as observed in some hereditary (e.g., hereditary angioedema caused by C1-esterase inhibitor deficiency—C1-INH-HAE) and acquired forms of recurrent angioedema (e.g., acquired angioedema with C1-inhibitor deficiency—C1-INH-AAE) [2] or a slow-down in BK degradation subsequent to the use of angiotensin-converting enzyme inhibitors (ACEI).

All the forms of BK-AE are characterized by recurrent episodes of edema in the subcutaneous or submucosal tissue, generally affecting the skin, gastrointestinal tract, and upper airways (these latter may be life-threatening) and resolving in 48-96 hours [2].

Among BK-AE, C1-INH-HAE is a rare autosomal dominant disease (ORPHA: 91378)¹, with a prevalence in Italy calculated to be equivalent to 1:64,935 [3]. The pathogenesis arises from the mutation of the SERPING1 gene, encoding for C1-INH. Type I C1-INH-HAE, detectable in 87% of Italian patients [3], is characterized by reduced plasma levels of C1-INH, while in type II, plasma concentrations are normal, but C1-INH activity is decreased.

ACEI-AAE is another form of BK-AE without wheals characterized by recurrent episodes of swelling that can affect any area of the body, but usually involve the face and upper airways. Diagnosis is based on exclusion of other causes of angioedema in patients taking ACEI.

¹Orpha.net. Hereditary Angioedema. Last update: August 2011. Available at: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=en&Expert=91378. Accessed February 7, 2019
New findings in recent years suggest that in all the forms of BK-AE, angioedema attacks result from increased levels of BK, highlighting the central role played by vessels in the pathogenesis of attacks. In fact, bradykinin binds to its receptor on endothelial cells, thus resulting in vascular leakage through pathways also involving vascular endothelial cadherin (VE-cadherin), endothelial nitric oxide synthase (eNOS), and vascular endothelial growth factor (VEGF)-A.

Recently, our group found that C1-INH-HAE patients have elevated plasma levels of VEGF-A, VEGF-C, Ang1, Ang2, and secreted phospholipase A₂ (sPLA₂) [4,5], thus supporting the hypothesis that the endothelium plays a pivotal role in the pathogenesis of C1-INH-HAE. Nailfold videocapillaroscopy (NVC) is a technique that studies structural characteristics of small vessels and it is used primarily in connective tissue diseases and in disorders involving vessels as pivotal pathogenic components, such as diabetes and arterial hypertension [6]. To date, there are no data on possible structural alterations of the vessels in angioedema patients. We hypothesized that, in C1-INH-HAE patients, minimal structural alterations in the vascular endothelium may exist and induce a state of “vascular preconditioning” predisposing patients to angioedema. In this case, the endothelium may represent a new therapeutic target in these patients. Therefore, the aim of this study was to evaluate the vessel features in angioedema patients during a symptom-free period using NVC. We also assessed the possible correlation of vessel characteristics with plasma levels of vascular permeability factors and with the angioedema attack.
Methods

Patients

We studied C1-INH-HAE and ACEI-AAE patients followed at the University of Naples Federico II during the period from June 2017 to June 2018 who were willing to participate. Diagnosis of C1-INH-HAE was based on the presence of at least one clinical and laboratory criteria as described by Cicardi et al. [2]. In particular, patients were diagnosed with C1-INH-HAE if they had recurrent angioedema attacks, low C4 levels, and C1-INH levels below 50% of the normal values. However, in case of normal C1-INH levels, C1-INH activity in plasma was also measured, as 15% of patients are affected by type II C1-INH-HAE, i.e. the mutated gene produces a dysfunctional protein. ACEI-AAE was diagnosed in case of onset of angioedema that could not be otherwise explained in patients taking ACEI. Subjects without angioedema were also enrolled, specifically healthy persons as controls for C1-INH-HAE and hypertensive patients (not necessarily taking ACEI) as controls for ACEI-AAE patients.

Subjects were excluded if they were affected by concomitant autoimmune pathologies, such as systemic lupus erythematosus, systemic sclerosis, arthritis, and Raynaud phenomenon. Using a specific form, the following data were collected: age, gender, ethnicity, familiar anamnesis, remote pathological anamnesis, history of the present illness, possible comorbidities (such as arterial hypertension, diabetes mellitus, and hypo/hyperthyroidism), data about angioedema attacks (frequency, duration, site, prophylaxis).

The clinical severity of C1-INH-HAE was classified both according to attack frequency (≥ or < 12 attacks in the preceding 12 months) and using the severity score proposed by Bygum et al. [7], based on age at onset, attack site, and the need for long-term prophylaxis.
NVC

NVC (VideoCap® 3.0-D1, DS Medica, Milan, Italy) is a highly sensitive, inexpensive, simple, safe, and noninvasive imaging technique used in the morphological analysis of nourishing capillaries in the nailfold area [8].

It is performed by means of a microscope combined with a digital video camera put in direct contact with a nailfold [9].

The patients under investigation should initially remain seated in an acclimatized room for 15-20 min, with a set temperature of approximately 22-23°C, in order to slow down blood flow, also eliminating the possible influence of climate on NVC results. A drop of immersion (cedar) oil was placed onto the cuticle of the fingers to be analyzed in order to reduce any refractive defects and improve capillary visualization [9]. Patients were instructed not to remove their fingernail cuticles for one month to avoid microtraumas that could put the examination at risk.

NVC was performed on the 4 fingers of both hands (excluding thumbs) with 200× magnification [6]. Angioedema patients underwent the procedure during a remission period (at least 8 days after an attack). NVC was also performed in two C1-INH-HAE patients during hand and abdominal attacks, respectively.

The following quantitative parameters were analyzed:

- Intercapillary distance: distance between two neighboring capillary loops, measured at the widest intercapillary space on the central capillary region;
- Apical diameter: distance from one external margin of the capillary loop to another on the apex (normal [8-25 µm], enlarged loop [>26 µm] or giant loop [>50 µm]);
- Loop length: «the distance between the apex of a capillary loop and the point where the capillary is no longer visible» [9];
Internal diameter: the distance between the efferent and the afferent loop measured at the same level;

External diameter: «the width of a capillary at its widest section» [9];

Capillary density: «number of capillaries in a 1-mm length of the distal row of each finger» [9].

The following qualitative parameters were analyzed:

- Capillary distribution: organization of capillaries, scored as ordered (0), comma-like (1), irregular (2), and severely deranged (3) [10];
- Capillary morphology: the aspect of capillaries, scored as hairpin-like (0), mainly tortuous (1), mainly ramified (2), and severe alterations (3) [10].

**Plasma collection**

Blood was collected during routine diagnostic procedures, and the remaining plasma sample was labeled with a code, which was documented into a datasheet. The controls had been referred for routine medical check-up and volunteered for the study by providing informed consent. Technicians who performed the assays were blinded to the patients’ history. The samples were collected by means of a clean venipuncture and minimal stasis using 3.2% sodium citrate. After centrifugation (2000 g for 20 min at 22°C), the plasma was divided into aliquots and stored at -80°C until usage. Blood samples from all patients were obtained at least 8 days apart from an angioedema attack (remission sample).

**Determination of VEGFs and Angiopoietins**

Plasma levels of angiogenic and lymphangiogenic mediators were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits for VEGF-A, VEGF-C, Ang1, and Ang2 (R&D System, Minneapolis, Minnesota, USA) according to the manufacturer's instructions. The
ELISA sensitivity is 31.1-2,000 pg/ml for VEGF-A, 62.5-4,000 pg/ml for VEGF-C, 156.25-10,000 pg/ml for Ang1, and 31.1-4,000 pg/ml for Ang2.

**Statistical Analyses**

Data were analyzed with the GraphPad Prism 5 software package. Data were tested for normality using the D’Agostino-Pearson normality test. If normality was not rejected at the 0.05 significance level, we used parametric tests. Otherwise, for non-normally distributed data we used nonparametric tests. Statistical analysis was performed by unpaired two-tailed t-test or two-tailed Mann-Whitney test. Correlations between two variables were assessed by Spearman’s correlation analysis and reported as coefficient of correlation (r). Capillaroscopic parameters are shown as the median (horizontal black line), the 25th and 75th percentiles (boxes), and the 5th and 95th percentiles (whiskers) of controls and patients. Statistically significant differences were accepted when the P value was ≤ 0.05.

**Ethical Aspects**

On November 7, 2018, the local ethics committee “Comitato Etico Federico II” approved with protocol number PT 1553/18 that plasma obtained during routine diagnostics could be used for research investigating the pathophysiology of angioedema, and written informed consent was obtained from every subject involved in this study according to the principles expressed in the Declaration of Helsinki.
Results

C1-INH-HAE Patients and Capillaroscopic Parameters

Thirty-four C1-INH-HAE patients and 28 healthy controls underwent NVC (Table 1). C1-INH-HAE patients showed significantly increased apical (median 28 µm [interquartile range (IQR) 23-26 µm] vs 22 µm [IQR 16-29 µm]) (Figure 1A), internal (median 22 µm [IQR 20-25 µm] vs 20 µm [IQR 18-22 µm]) (Figure 1B), and external diameters (median 81 µm [IQR 65-91 µm] vs 65 µm [IQR 55-73 µm]) (Figure 1C) compared to healthy controls, thus demonstrating the presence of enlarged capillaries. By contrast, loop length did not show any difference between the two groups (median 450 µm [IQR 330-556 µm] vs 480 µm [IQR 350-590 µm]) (Figure 1D). In addition, both significantly increased intercapillary distance (median 216 µm [IQR 185-253 µm] vs 190 µm [IQR 154-236 µm]) (Figure 1E) and decreased capillary density (median 4/mm [IQR 4-5/mm] vs 5/mm [IQR 5-6/mm]) (Figure 1F) indicate a reduction in the number of capillaries in C1-INH-HAE patients compared to controls. Capillary distribution was significantly more irregular (median 0 [IQR 0-1] vs 0 [IQR 0-0]) (Figure 1G) and the morphology mainly tortuous (median 1 [IQR 0-1] vs 0 [IQR 0-0]) (Figure 1H) in C1-INH-HAE patients versus controls. Figure 2A-C shows representative images of capillaroscopic parameters of three different patients with C1-INH-HAE. In particular, Figure 2A shows increased apical, internal, and external diameters, Figure 2B shows reduced capillary density, and Figure 2C shows irregular capillary distribution. With the aim of identifying which parameters changed together, we assessed the presence of proportionality among them, finding that several correlations among capillaroscopic parameters in C1-INH-HAE patients further support the presence of multiple alterations in capillaries. In fact, direct proportionality was found among loop length, intercapillary distance, and apical diameter (Figure 3A-C). By contrast, apical diameter, loop length, and external
diameter were inversely correlated with capillary density (Figure 3D-F). In addition, capillary distribution was directly proportional to capillary density and morphology (data not shown).

Among healthy subjects, no significant correlations were found between capillaroscopic parameters and age or BMI. On the contrary, intercapillary distance was significantly different according to gender: females have greater intercapillary distance ($P < 0.05$). Interestingly, C1-INH-HAE patients also had significant alterations according to gender, but with an overturned correlation: males have greater intercapillary distance, apical diameter, loop length, and external diameter, and more altered capillary distribution (Figure 4).

**C1-INH-HAE Characteristics and Capillaroscopic Parameters**

To evaluate whether NVC findings in C1-INH-HAE patients were related to pathology severity, we assessed the presence of correlations among capillaroscopic parameters and the severity score proposed by Bygum et al. [7]: no significant associations were found (Figure 5).

However, a significant correlation was found with the frequency of attacks: C1-INH-HAE patients with $\geq 12$ attacks/year (17/34 patients) had greater apical diameter with respect to those having $< 12$ attacks/year (Figure 6A). By contrast, other capillary characteristics were not correlated with the severity of disease (Figure 6B-H).

No significant differences in capillaroscopic parameters were found between patients in long-term prophylaxis—LTP (i.e. regularly taking plasma-derived C1-INH or androgens) and patients not under LTP (data not shown).

Similarly, the age of onset of C1-INH-HAE symptoms seemed to have no role in the onset of capillary alterations, as no significant correlations were found (data not shown).
Vasoactive Mediators and Capillaroscopic Parameters

Finally, to test the hypothesis that capillaroscopic parameters change in accordance with mediators known to regulate vascular permeability, we assessed whether capillaroscopic alterations were correlated with plasma levels of VEGF-A, VEGF-C, Ang1, and Ang2, but no significant correlations were found (Figure 7, 8, 9, and 10).

Capillaroscopic Parameters in Remission and During Attacks

A further exploratory analysis on a very small sample tested whether capillary alterations changed between remission and attack phases: NVC performed in only two C1-INH-HAE patients during hand and abdominal attacks, respectively, showed no changes compared to the remission phase (Figure 11A-H).

Capillaroscopic Parameters in ACEI-AAE Patients and Their Controls

In a final series of experiments, we checked whether an acquired type of BK-AE had the same capillaroscopic alterations found in C1-INH-HAE patients.

Sixteen patients affected by ACEI-AAE (median age = 72.5 years, range: 46-82; 7 female patients) were also recruited, with 7 hypertensive subjects with similar comorbidities, but not affected by ACEI-AAE (median age = 65 years, range: 42-78; 1 female subject).

In ACEI-AAE patients, videocapillaroscopy showed no alterations. In particular, quantitative parameters of ACEI-AE did not show any difference with respect to hypertensive controls, i.e., apical (median 22 µm [IQR 19-25 µm] vs 20 µm [IQR 16-25 µm]) (Figure 12A), internal (median 19 µm [IQR 15-21 µm] vs 20 µm [IQR 18-20 µm]) (Figure 12B), and external diameters (median 81 µm [IQR 65-91 µm] vs 70 µm [IQR 60-75 µm]) (Figure 12C), loop length (median 441 µm [IQR 370-387
μm] vs 424 μm [IQR 250-723 μm]) (Figure 12D), intercapillary distance (median 188 μm [IQR 150-277 μm] vs 160 μm [IQR 130-200 μm]) (Figure 12E), and capillary density (median 5/mm [IQR 5-6/mm] vs 6/mm [IQR 5-7/mm]) (Figure 12F). Likewise, qualitative parameters, i.e., capillary distribution (median 0 [IQR 0-1] vs 0 [IQR 0-1]) (Figure 12G) and morphology (median 1 [IQR 1-1] vs 1 [IQR 0-1]) (Figure 12H) were similar to controls.
Discussion

This is the first study evaluating the structural alterations of capillaries in patients with angioedema. Patients affected by C1-INH-HAE have capillaries characterized by significant quantitative and qualitative morphological alterations, i.e., increased apical, internal, and external diameters, reduced capillary density, and increased prevalence of irregular capillary distribution and tortuous morphology. A derangement of capillary structure was observed in these patients: capillaries become progressively larger and less numerous.

It remains unknown whether those alterations trigger attacks or if, on the contrary, are caused by attacks. If the first hypothesis is confirmed by other studies, the pathogenesis of angioedema will be further elucidated as a kind of “vascular preconditioning” may predispose patients to angioedema attacks, and, consequently, the endothelium may become a new therapeutic target.

The presence of altered capillaries characterizes C1-INH-HAE patients. Nailfold videocapillaroscopy was performed during acute attacks just in two patients, who showed no significant changes compared to the basal conditions. Obviously, it is not possible to draw conclusions from such a small sample. However, it should be pointed out that it is difficult to perform NVC during acute attacks. Therefore, our preliminary data may suggest that such alterations are constitutive characteristics of C1-INH-HAE patients, which are not associated with the acute phase, but obviously a larger sample size would be necessary in order to confirm this hypothesis.

Some published studies report results regarding the presence of endothelial dysfunction in C1-INH-HAE patients.

We previously hypothesized that such patients have a sort of “vascular preconditioning” that may predispose them to angioedema attack due to the detection of higher plasma levels of angiogenic
factors (VEGF-A, VEGF-C, Ang1, and Ang2) in 128 C1-INH-HAE patients in comparison with 68 healthy subjects [4]. This hypothesis was further corroborated by the higher plasma levels of VEGF-A, VEGF-C, and Ang2 detected in patients with ≥ 12 attacks/year.

Even though in the present study capillaroscopic alterations were not correlated with plasma levels of VEGF-A, VEGF-C, Ang1, and Ang2, the results obtained in terms of capillary differences led to the same conclusion about the presence of vascular preconditioning, as all except one (i.e., loop length) among the parameters analyzed were found to be significantly different with respect to controls. Due to the types of procedures used, it may be necessary to focus on the evaluation of histological aspects of the endothelium rather than on serum factors. We hypothesize that future studies, involving larger sample sizes, may show significant correlations between angiogenic and lymphangiogenic mediators and morphologic vascular changes.

An endothelial dysfunction in hereditary angioedema (both due to C1-INH deficiency and factor XII deficiency) was also found by Firinu et al. [11], who detected a lower reactive hyperemia index with non-invasive finger plethysmography in the fingertips in 24 HAE patients compared with 24 age- and gender-matched healthy controls. HAE patients also had higher plasma levels of asymmetric dimethylarginine (ADMA). However, these alterations had no correlations with disease severity.

On the contrary, the study of Nebenführ et al. [12] excluded the presence of endothelial dysfunction in a study involving 33 C1-INH-HAE patients and 30 healthy controls. The technique used in that case was the flow-mediated dilation method measured at the brachial artery. The contrasting conclusions with respect to the study of Firinu et al. may arise from the different measurement techniques used and the heterogeneity of patient populations recruited [13].

Finally, the presence of many tortuous loops in capillaries was also found by means of NVC in a family affected by unknown hereditary angioedema (U-HAE) [14].
In our study, in patients affected by a different form of bradykinin-mediated angioedema, i.e., ACEI-AAE, neither qualitative (irregular distribution and presence of tortuous capillaries) nor quantitative alterations were detected. These findings may support the hypothesis that capillary alterations represent a feature of hereditary angioedema.

Among capillaroscopic parameters, apical diameter may be a severity predictor, as C1-INH-HAE patients with the most severe forms of angioedema (those having ≥12 attacks/year) had a significantly greater apical diameter compared to those with < 12 attacks/year.

A partial explanation may come from statistical analyses, which showed that an increase in apical diameter correlates with an increase in intercapillary distance, external diameter, and capillary length and a decrease in capillary density, suggesting a greater severity of alterations.

C1-INH-HAE patients had significant alterations according to gender, but their significance has yet to be understood. On the contrary, among healthy subjects, the only gender-related significant alteration was the greater intercapillary distance found in females. A greater intercapillary distance for girls compared to boys ($P = 0.011$) was also the only significant alteration found by Piotto et al., who performed NVC in 100 healthy subjects aged 5 to 18 years [15].

Data collected in our healthy subjects may contribute to the determination of normal values of capillaroscopic parameters, since in the literature, they are scarce. Our data are generally in line with those reported in the study carried out on the greatest sample size of healthy subjects [16].

NVC seems to be the most suitable method to analyze the endothelium in angioedema patients due to its features: it is simple to perform, non-invasive, repeatable, inexpensive, and highly sensitive. To our knowledge, this is the first study which uses NVC in hereditary angioedema. Therefore, we referred to the data in the literature in the rheumatology field to design the protocol and measure capillaroscopic parameters. Other working groups in the rheumatology field consider NVC a useful prognostic and
diagnostic tool [17], with a high reliability, regardless of the clinicians’ experience [18]. The nail bed is analyzed because of the easy approach with the probe and the fact that in this site, capillary vessels are parallel to the cutaneous surface [6].

This study has some limitations. First, the sample size was low. This is a common issue when a rare disease is considered. Second, even though most characteristics of patients and healthy subjects were similar, some of them were not (i.e., gender in the comparison ACEI-AAE patients/controls). However, we do not think these discrepancies affect the reliability of our results. Third, parameters were measured during attack in only two patients, thereby making the resulting measures less reliable. Due to the unpredictability of attack onset, it is not possible to plan such a procedure in the acute phase of this pathology.

In conclusion, our data suggest that C1-INH-HAE patients have important structural alterations of the capillaries, confirming the presence of an endothelial dysfunction in the angioedema. The absence of significant capillaroscopic alterations in patients with ACEI-AAE suggests that these findings may be related to the pathogenesis of hereditary angioedema. This pilot study, performed on a small sample size, has limited clinical value, but may open the doors for future larger studies, that will help widen the knowledge on the underlying mechanisms of C1-INH-HAE and identify possible therapeutic targets.

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Previous presentation

The main results of this study were presented as poster at the “European Academy of Allergy and Clinical Immunology (EAACI) Congress” held in Munich (Germany) in 2018 and in Lisbon (Portugal) in 2019, and at the “5th International Conference of translational medicine on pathogenesis and therapy of immunomediared diseases. Innate immunity, inflammation and experimental models of human diseases” held in Milan (Italy) in 2019.

The study was also presented in the oral session at the “Bradykinin Symposium” held in Berlin (Germany) in 2018.

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Conflicts of Interest

Dr. Cesoni Marcelli reports non-financial support from CSL Behring, during the conduct of the study; Dr. Loffredo reports non-financial support from CSL-Behring, during the conduct of the study; grants, personal fees and non-financial support from Takeda (Shire), non-financial support from CSL-Behring, outside the submitted work; Dr. Petraroli reports non-financial support from CSL Behring, during the conduct of the study; other from CSL Behring, other from Shire, other from Allergic Therapeutics, personal fees from Shire, other from Novartis, other from AstraZeneca, outside the submitted work; Dr. Carucci reports non-financial support from CLS Behring, during the conduct of
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References

1. Cicardi M, Zuraw BL. Angioedema Due to Bradykinin Dysregulation. J Allergy Clin Immunol Pract. 2018;6:1132-41.

2. Cicardi M, Aberer W, Banerji A, Bas M, Bernstein JA, Bork K, et al.; HAWK under the patronage of EAACI (European Academy of Allergy and Clinical Immunology). Classification, diagnosis, and approach to treatment for angioedema: consensus report from the Hereditary Angioedema International Working Group. Allergy. 2014;69:602-16.

3. Zanichelli A, Arcoleo F, Barca MP, Borrelli P, Bova M, Cancian M, et al. A nationwide survey of hereditary angioedema due to C1 inhibitor deficiency in Italy. Orphanet J Rare Dis. 2015;10:11.

4. Loffredo S, Bova M, Suffritti C, Borriello F, Zanichelli A, Petraroli A, et al. Elevated plasma levels of vascular permeability factors in C1 inhibitor-deficient hereditary angioedema. Allergy. 2016;71:989-96.

5. Loffredo S, Ferrara AL, Bova M, Borriello F, Suffritti C, Veszeli N, et al. Secreted Phospholipases A2 in Hereditary Angioedema With C1-Inhibitor Deficiency. Front Immunol. 2018;9:1721.

6. Gallucci F, Russo R, Buono R, Acampora R, Madrid E, Uomo G. Indications and results of videocapillaroscopy in clinical practice. Adv Med Sci. 2008;53:149-57.

7. Bygum A, Fagerberg CR, Ponard D, Monnier N, Lunardi J, Drouet C. Mutational spectrum and phenotypes in Danish families with hereditary angioedema because of C1 inhibitor deficiency. Allergy. 2011;66:76-84.
8. Lambova SN, Müller-Ladner U. Capillaroscopic pattern in systemic lupus erythematosus and undifferentiated connective tissue disease: what we still have to learn? Rheumatol Int. 2013;33:689-95.

9. Etehad Tavakol M, Fatemi A, Karbalaie A, Emrani Z, Erlandsson BE. Nailfold Capillaroscopy in Rheumatic Diseases: Which Parameters Should Be Evaluated? Biomed Res Int. 2015;2015:974530.

10. Hsu PC, Liao PY, Chang HH, Chiang JY, Huang YC, Lo LC. Nailfold capillary abnormalities are associated with type 2 diabetes progression and correlated with peripheral neuropathy. Medicine (Baltimore). 2016;95:e5714.

11. Firinu D, Bassareo PP, Zedda AM, Barca MP, Crisafulli A, Mercuro G, et al. Impaired Endothelial Function in Hereditary Angioedema During the Symptom-Free Period. Front Physiol. 2018;9:523.

12. Nebenführer Z, Szabó E, Kajdácsi E, Kőhalmi KV, Karádi I, Zsáry A, et al. Flow-mediated vasodilation assay indicates no endothelial dysfunction in hereditary angioedema patients with C1-inhibitor deficiency. Ann Allergy Asthma Immunol. 2019;122:86-92.

13. Bassareo PP, Firinu D, Del Giacco S. Is hereditary angioedema related to an increased risk of atherosclerosis? Ann Allergy Asthma Immunol. 2019;122:228-9.

14. Bafunno V, Firinu D, D’Apolito M, Cordisco G, Loffredo S, Leccese A, et al. Mutation of the angiopoietin-1 gene (ANGPT1) associates with a new type of hereditary angioedema. J Allergy Clin Immunol. 2018;141:1009-17.

15. Piotto DP, Sekiyama J, Kayser C, Yamada M, Len CA, Terreri MT. Nailfold videocapillaroscopy in healthy children and adolescents: description of normal patterns. Clin Exp Rheumatol. 2016;34 Suppl 100(5):193-9.
16. Ingegnoli F, Gualtierotti R, Lubatti C, Bertolazzi C, Gutierrez M, Boracchi P, et al. Nailfold capillary patterns in healthy subjects: a real issue in capillaroscopy. Microvasc Res. 2013;90:90-5.

17. Ocampo-Garza SS, Villarreal-Alarcón MA, Villarreal-Treviño AV, Ocampo-Candiani J. Capillaroscopy: A Valuable Diagnostic Tool. Actas Dermosifiliogr. 2019;110:347-352.

18. Cutolo M, Melsens K, Herrick AL, Foeldvari I, Deschepper E, De Keyser F, et al.; EULAR Study Group on Microcirculation in Rheumatic Diseases. Reliability of simple capillaroscopic definitions in describing capillary morphology in rheumatic diseases. Rheumatology (Oxford). 2018;57:757-759.
Table 1. Characteristics of C1-INH-HAE patients and healthy controls.

|                              | Healthy Controls (n = 28) | C1-INH-HAE Patients (n = 34) |
|------------------------------|---------------------------|-----------------------------|
| Age, years (median, range)   | 40 (9-72)                 | 39.5 (9-74)                 |
| Female gender (n, %)         | 14 (50%)                  | 17 (50%)                    |
| Caucasian ethnicity (%)      | 100%                      | 100%                        |
| BMI (median, range)          | 24 (21-26)†               | 23.4 (15-40)                |
| Hypertension (n, %)          | 1 (7.7%)†                 | 3 (9%)                      |
| Dysthyroidism (n, %)         | 0 (0%)†                   | 1 (3%)                      |
| Diabetes Mellitus (n, %)     | 0 (0%)†                   | 0 (0%)                      |
| Type I C1-INH-HAE            | -                         | 31 (91.2%)                  |
| Type II C1-INH-HAE           | -                         | 3 (8.8%)                    |
| Severity score (median, IQR) | -                         | 7 (4.7-8)                   |
| Attack frequency (≥ 12 attacks/year, %) | -                           | 17 (50%)                   |
| Age at onset (median, range) | -                         | 6 (1-22)†                  |
| Long-term prophylaxis        |                           |                             |
| No long-term prophylaxis (n, %) | -                         | 23 (67.6%)                   |
| Attenuated androgens        | -                         | 6 (17.6%)                    |
| C1-inhibitor                | -                         | 5 (14.7%)                    |
| Therapy for acute attacks    |                           |                             |
### Plasma-derived C1-inhibitor  
-  
27 (79.4%)

### Icatibant  
-  
7 (20.6%)

#### Attack site

| Attack Site                  | (n, %) | Count (%) |
|------------------------------|--------|-----------|
| Skin                         | -      | 32 (94.2%)|
| Gastrointestinal tract       | -      | 27 (79.5%)|
| Larynx                       | -      | 19 (56%)  |
| Genitalia                    | -      | 8 (23.5%) |

**BMI**, Body mass index; **C1-INH-HAE**, C1-inhibitor hereditary angioedema; **IQR**, interquartile range

† Data available for 13 subjects

‡ Thirty-two patients were considered, as two of them were asymptomatic
Figure legends

Figure 1. Capillaroscopic parameters in C1-INH-HAE patients and healthy controls (A-H).
Apical (A), internal (B), external diameters (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) were measured in 34 C1-INH-HAE patients in the symptom-free period and 28 healthy controls. Horizontal bars depict the median value (A-H), boxes the 25th and 75th percentiles, and whiskers the 5th and 95th percentiles (A-F).
Figure 2. Images from videocapillaroscopy (200× magnification) on recruited C1-INH-HAE patients showing apical (AD), internal (ID), and external (ED) diameters (A); capillary density (B); capillary distribution (C).
Figure 3. Correlations between two capillaroscopic parameters in C1-INH-HAE patients.

Apical diameter and intercapillary distance (A), loop length and intercapillary distance (B), loop length and apical diameter (C), density and apical diameter (D), density and loop length (E), and density and external diameter (F) were assessed by Spearman’s correlation analysis and reported as coefficient of correlation (r).
Figure 4. Relationship between capillaroscopic parameters and gender of C1-INH-HAE patients. Apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) were determined in 17 males and 17 females. Data (A-H) are shown as the median (horizontal black line), the 25th and 75th percentiles (boxes), and the 5th and 95th percentiles (whiskers) of 34 C1-INH-HAE patients for capillaroscopic parameters. *P* value ≤ 0.05 was considered significant.
Figure 5. Correlations between capillaroscopic parameters and severity of disease.

The correlation between apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) and severity score were assessed by Spearman’s correlation analysis and reported as coefficient of correlation (r). $P$ value ≤ 0.05 was considered significant.
Figure 6. Relationship between capillaroscopic parameters and frequency of attacks in C1-INH-HAE patients.

Apical (A), internal (B), external diameters (C), loop length (D), intercapillary distance (E), density (F), distribution (G), and morphology (H) were determined in 17 patients with low (<12/year) and 17 patients with high frequency (≥12/year). Horizontal bars depict the median value (A-H), boxes the 25th and 75th percentiles, and whiskers the 5th and 95th percentiles of 34 C1-INH-HAE patients.
Figure 7. Correlations between capillaroscopic parameters and plasma concentrations of VEGF-A. The correlation between apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) and VEGF-A levels (measured by ELISA) in C1-INH-HAE patients were assessed by Spearman’s correlation analysis and reported as coefficient of correlation (r). $P$ value $\leq 0.05$ was considered significant.
Figure 8. Correlations between capillaroscopic parameters and plasma concentrations of VEGF-C. The correlation between apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) and VEGF-C levels (measured by ELISA) in C1-INH-HAE patients were assessed by Spearman’s correlation analysis and reported as coefficient of correlation (r). P value ≤ 0.05 was considered significant.
Figure 9. Correlations between capillaroscopic parameters and plasma concentrations of Ang1. The correlation between apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) and Ang1 levels (measured by ELISA) in C1-INH-HAE patients were assessed by Spearman’s correlation analysis and reported as coefficient of correlation (r). P value ≤ 0.05 was considered significant.
Figure 10. Correlations between capillaroscopic parameters and plasma concentrations of Ang2. The correlation between apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) and Ang2 levels (measured by ELISA) in C1-INH-HAE patients were assessed by Spearman’s correlation analysis and reported as coefficient of correlation (r). \( P \) value \( \leq 0.05 \) was considered significant.
Figure 11. Capillaroscopic parameters in remission and attack periods in C1-INH-HAE patients. Apical (A), internal (B), external diameters (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) were determined in 2 patients with C1-INH-HAE in symptom-free period and during angioedema attack. Squares indicate data about the patient experiencing a gastrointestinal attack, while circles indicate data about the patient experiencing a hand attack.
Figure 12. Capillaroscopic parameters in ACEI-AAE patients and healthy controls.

Apical (A), diameter (B), external diameters (C), loop length (D), intercapillary distance (E), density (F), distribution (G), and morphology (H) were measured in 16 ACEI-AAE patients in the symptom-free period and 7 hypertensive controls. Horizontal bars depict the median value (A-H), boxes the 25\textsuperscript{th} and 75\textsuperscript{th} percentiles, and whiskers the 5\textsuperscript{th} and 95\textsuperscript{th} percentiles (A-F).