ORIGINAL ARTICLE

Nailfold Videocapillaroscopy Findings in Bradykinin-Mediated Angioedema

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■ Abstract

Background: Hereditary angioedema with C1-inhibitor deficiency (C1-INH-HAE) and acquired angioedema related to angiotensin-converting enzyme (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, although no data are available on possible structural alterations of the vessels.

Objective: This study aims to compare the features of nailfold capillaries to highlight possible structural anomalies between patients affected by C1-INH-HAE and controls and between patients with ACEI-AAE and hypertensive controls.

Methods: We used nailfold videocapillaroscopy (NVC) to assess the following: apical, internal, and external diameter; loop length; intercapillary distance; and 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: Compared with healthy controls (n=28), C1-INH-HAE patients (n = 34) were characterized by significant structural alterations of the capillaries, such as 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/mm^2), more irregular capillary distribution, and more tortuous morphology. Apical diameter was enlarged in patients with ≥12 attacks per year. In ACEI-AAE patients, NVC showed no alterations with respect to hypertensive controls. NVC performed in 2 C1-INH-HAE patients during attacks showed no changes compared with the remission phase.

Conclusions: We detected major structural capillary alterations in C1-INH-HAE patients, thus confirming the involvement of microcirculation in the pathogenesis of angioedema.

Key words: 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 "preacondicionamiento 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 videocapilaroscopia 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/mm^2), 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. Preacondicionamiento 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 [1].

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

All the forms of BK-AE are characterized by recurrent episodes of edema in the subcutaneous or submucosal tissue. These generally affect the skin, gastrointestinal tract, and upper airways (potentially life-threatening in this case) and resolve in 48-96 hours [2].

Within BK-AE, C1-INH-HAE is a rare autosomal dominant disease (ORPHA: 91378) [3], whose prevalence in Italy is 1:64 935 [4]. The disease is caused by a mutation of the SERPING1 gene, which encodes C1-INH. Type I C1-INH-HAE, which is detectable in 87% of Italian patients [4], is characterized by reduced plasma levels of C1-INH, while type II disease is characterized by normal plasma concentrations and decreased C1-INH activity.

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 involves the face and upper airways. Diagnosis is based on exclusion of other causes of angioedema in patients taking ACEI.

Recent findings suggest that in all the forms of BK-AE, angioedema attacks result from increased levels of bradykinin, thus highlighting the central role played by vessels in the pathogenesis of attacks. In fact, bradykinin binds to its receptor on endothelial cells, resulting in vascular leakage through pathways also involving vascular endothelial 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, angiopoietin (Ang1), Ang2, and secreted phospholipase A2 [5,6], thus supporting the hypothesis that the endothelium plays a pivotal role in the pathogenesis of C1-INH-HAE. Nailfold videocapillaroscopy (NVC) makes it possible to study the structural characteristics of nailfold small vessels and is used primarily in connective tissue diseases and in disorders involving vessels as pivotal pathogenic components, such as diabetes and arterial hypertension [7]. No data have been reported 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 can induce a state of "vascular preconditioning" that predisposes patients to angioedema. In this case, the endothelium may represent a new therapeutic target in affected patients. Therefore, we used NVC to evaluate the features of vessels in angioedema patients during a symptom-free period. We also assessed the possible correlation between vessel characteristics, plasma levels of vascular permeability factors, and the angioedema attack.

Methods

Patients

We studied C1-INH-HAE and ACEI-AAE patients followed at University of Naples Federico II between June 2017 and June 2018 who agreed to participate in the study. Diagnosis of C1-INH-HAE was based on the presence of at least 1 clinical and 1 laboratory criterion as described by Cicardi et al [2]. Patients were diagnosed with C1-INH-HAE if they had recurrent angioedema attacks, low C4 levels, and C1-INH levels below 50% of normal. However, in the 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, ie, the mutated gene produces a dysfunctional protein. A diagnosis of ACEI-AAE was made if angioedema could not be otherwise explained in patients taking ACEI. Participants without angioedema were also enrolled, specifically healthy persons to act as controls for C1-INH-HAE and hypertensive patients (not necessarily taking ACEI) to act as controls for ACEI-AAE patients.

Participants were excluded if they had concomitant autoimmune diseases, such as systemic lupus erythematosus, systemic sclerosis, arthritis, and Raynaud phenomenon. A series of data were collected using a specifically designed form, as follows: age, sex, ethnicity, family history, disease history, history of the present illness, possible comorbidities (such as arterial hypertension, diabetes mellitus, and hypo/hyperthyroidism), and angioedema attacks (frequency, duration, site, prophylaxis).

The clinical severity of C1-INH-HAE was classified both according to attack frequency ($\geq$ or <12 attacks in the preceding 12 months) and using the severity score proposed by Bygum et al [8], based on age at onset, attack site, and the need for long-term prophylaxis.

Nailfold Videocapillaroscopy

NVC (VideoCap 3.0-D1, DS Medica) is a highly sensitive, inexpensive, simple, safe, and noninvasive imaging technique used in the morphological analysis of capillaries in the nailfold area [9]. It comprises a microscope combined with a digital video camera placed in direct contact with a nailfold [10].

The patient under investigation should initially remain seated in an acclimatized room for 15-20 minutes at a set temperature of approximately 22-23°C in order to slow down blood flow and eliminate 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 with the aim of reducing refractive defects and improving visualization of the capillaries [10]. Patients were instructed not to remove their fingernail cuticles for 1 month to avoid microtraumas that could put the examination at risk.

NVC was performed on the 4 fingers of both hands (excluding the thumbs) at a ×200 magnification [7]. 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.

A series of quantitative parameters were analyzed, as follows:

– Intercapillary distance: distance between 2 neighboring capillary loops, measured at the widest intercapillary space in 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 [10]

– 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 [10]

– Capillary density: number of capillaries in a 1-mm length of the distal row of each finger [10]

The qualitative parameters analyzed were as follows:

– Capillary distribution: organization of capillaries, scored as ordered (0), comma-like (1), irregular (2), and severely deranged (3) [11]

– Capillary morphology: the appearance of capillaries, scored as hairpin-like (0), mainly tortuous (1), mainly ramified (2), and severe alterations (3) [11]

Plasma Collection

Blood was collected during routine diagnostic procedures, and the plasma sample was labeled with a code, which was entered into a datasheet. The controls had been referred for a routine medical check-up and gave their informed consent to participate. The technicians who performed the assays were blind to the patients' history. The samples were collected by means of venipuncture and minimal stasis using 3.2% sodium citrate. After centrifugation (2000g for 20 minutes 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 Systems) according to the manufacturer's instructions. The sensitivity of ELISA is 31.1-2000 pg/mL for VEGF-A, 62.5-4000 pg/mL for VEGF-C, 156.25-10 000 pg/mL for Ang1, and 31.1-4000 pg/mL for Ang2.

Statistical Analyses

Data were analyzed using GraphPad Prism 5. 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 nonnormally distributed data, we used nonparametric tests. Statistical analysis was performed using an unpaired 2-tailed t test or 2-tailed Mann-Whitney test. Correlations between 2 variables were analyzed using the Spearman correlation and reported as the correlation coefficient (r). Capillaroscopic parameters are shown as the median (horizontal black line), 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 ≤.05.

Ethics

The local ethics committee "Comitato Etico Federico II" authorized plasma obtained during routine diagnostics to be used for research investigating the pathophysiology of angioedema (date November 7, 2018; protocol number PT 1553/18). Written informed consent was obtained from all study participants according to the principles expressed in the Declaration of Helsinki.

Results

C1-INH-HAE Patients and Capillaroscopy Parameters

Thirty-four C1-INH-HAE patients and 28 healthy controls underwent NVC (Table).

Compared with healthy controls, C1-INH-HAE patients showed significantly increased apical diameter (median 28 [23-26] µm vs 22 [16-29] µm) (Figure 1A), internal diameter (median 22 [20-25] µm vs 20 [18-22] µm) (Figure 1B), and external diameter (median 81 [65-91] µm vs 65 [55-73] µm) (Figure 1C), thus demonstrating the presence of enlarged capillaries. By contrast, loop length did not differ between the 2 groups (median 450 [330-556] µm vs 480 [350-590] µm) (Figure 1D). In addition, the finding of significantly increased intercapillary distance (median 216 [185-253] µm vs 190 [154-236] µm) (Figure 1E) and decreased capillary density (median 4 [4-5] per millimeter vs 5 [5-6] per millimeter) (Figure 1F) indicates fewer capillaries in C1-INH-HAE patients than in controls. Capillary distribution was significantly more irregular (0 [0-1] vs 0 [0-0]) (Figure 1G) and the morphology mainly tortuous (1 [0-1] vs 0 [0-0]) (Figure 1H) in C1-INH-HAE patients than in controls. Figure 2A-C shows representative images for the capillaroscopy parameters of 3 different patients with C1-INH-HAE. 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 proportionality between them, finding that several correlations between capillaroscopy parameters in C1-INH-HAE patients further support the presence of multiple capillary abnormalities. In fact, the association between loop length, intercapillary distance, and apical diameter was directly proportional (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).

Table. Characteristics of C1-INH-HAE Patients and Healthy Controls

	Healthy controls (n=28)	C1-INH-HAE patients (n=34)
Median (range) age, y	40 (9-72)	39.5 (9-74)
Female sex, No. (%)	14 (50%)	17 (50%)
White, No. (%)	100%	100%
BMI (median, range)	24 (21-26)[a]	23.4 (15-40)
Hypertension, No. (%)	1 (7.7%)[a]	3 (9%)
Dysthyroidism, No. (%)	0 (0%)[a]	1 (3%)
Diabetes mellitus, No. (%)	0 (0%)[a]	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), No. (%)	-	17 (50%)
Age at onset, median (range)	-	6 (1-22)[b]
Long-term prophylaxis		
No long-term prophylaxis, No. (%)	-	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		
Skin, No. (%)	-	32 (94.2%)
Gastrointestinal tract, No. (%)	-	27 (79.5%)
Larynx, No. (%)	-	19 (56%)
Genitalia, No. (%)	-	8 (23.5%)

Abbreviations: BMI, body mass index; C1-INH-HAE, C1-inhibitor hereditary angioedema.
[a]Data available for 13 patients.
[b]Includes 32 patients, as 2 were asymptomatic.

Among healthy controls, no significant correlations were found between capillaroscopy parameters and age or body mass index. In contrast, intercapillary distance differed significantly by sex, with intercapillary distance greater in females (P < 0.05). Interestingly, C1-INH-HAE patients also differed significantly by sex, although with an inverse correlation: intercapillary distance, apical diameter, loop length, and external diameter were greater in males, and anomalies of capillary distribution were more pronounced (Figure 4).

Characteristics of C1-INH-HAE and Capillaroscopy Parameters

To evaluate whether NVC findings in C1-INH-HAE patients were related to disease severity, we assessed the presence of correlations between capillaroscopy parameters and the severity score proposed by Bygum et al [8]. No significant associations were found (Figure 5).

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

No significant differences in capillaroscopy parameters were found between patients under long-term prophylaxis (ie, regularly taking plasma-derived C1-INH or androgens) and patients not under long-term prophylaxis (data not shown).

Similarly, the absence of significant correlations indicates that the age at onset of C1-INH-HAE symptoms had no role in the onset of capillary abnormalities (data not shown).
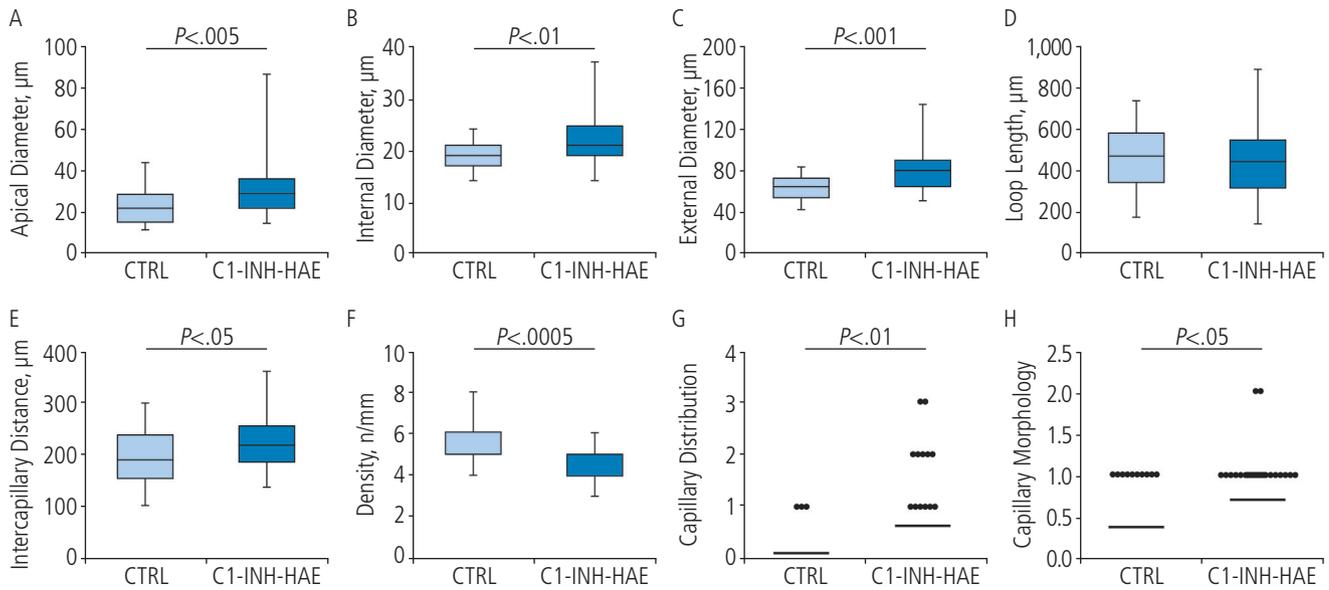
Figure 1. Capillaroscopic parameters in C1-INH-HAE patients and healthy controls (A-H). Apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) were measured in 34 C1-INH-HAE patients during the symptom-free period and in 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).
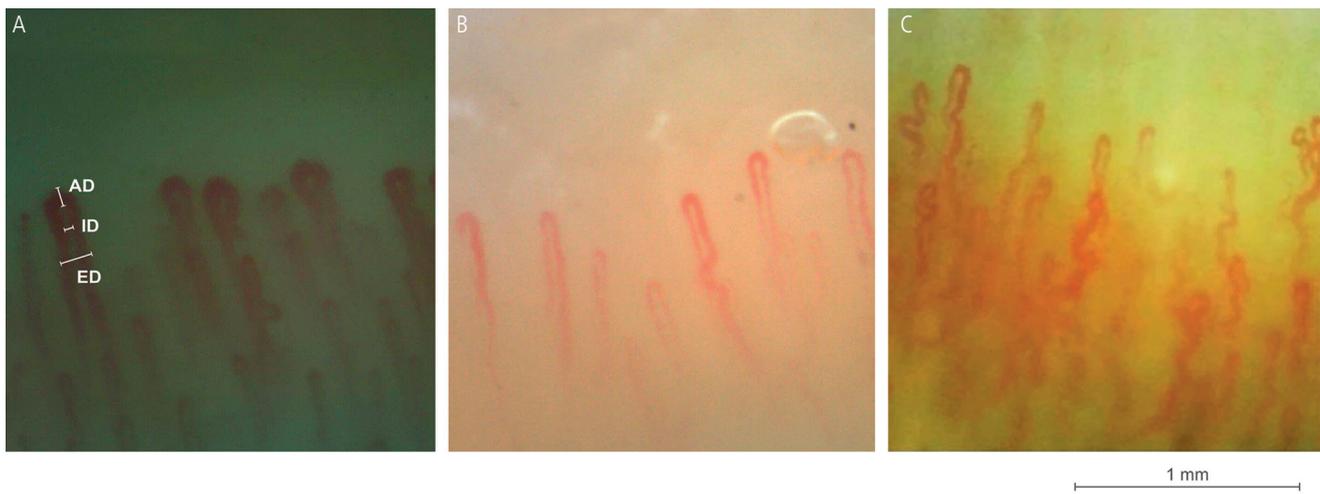

Figure 2. Images from videocapillaroscopy (magnification ×200) on recruited C1-INH-HAE patients showing apical (AD), internal (ID), and external (ED) diameters (A), as well as capillary density (B) and capillary distribution (C).

Vasoactive Mediators and Capillaroscopy Parameters

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

Capillaroscopy Parameters in Remission and During Attacks

A further exploratory analysis of a very small sample tested whether capillary alterations changed between the remission and attack phases: NVC performed in only 2 C1-INH-HAE patients during hand and abdominal attacks, respectively, showed no changes compared with the remission phase (Figure 11A-H).
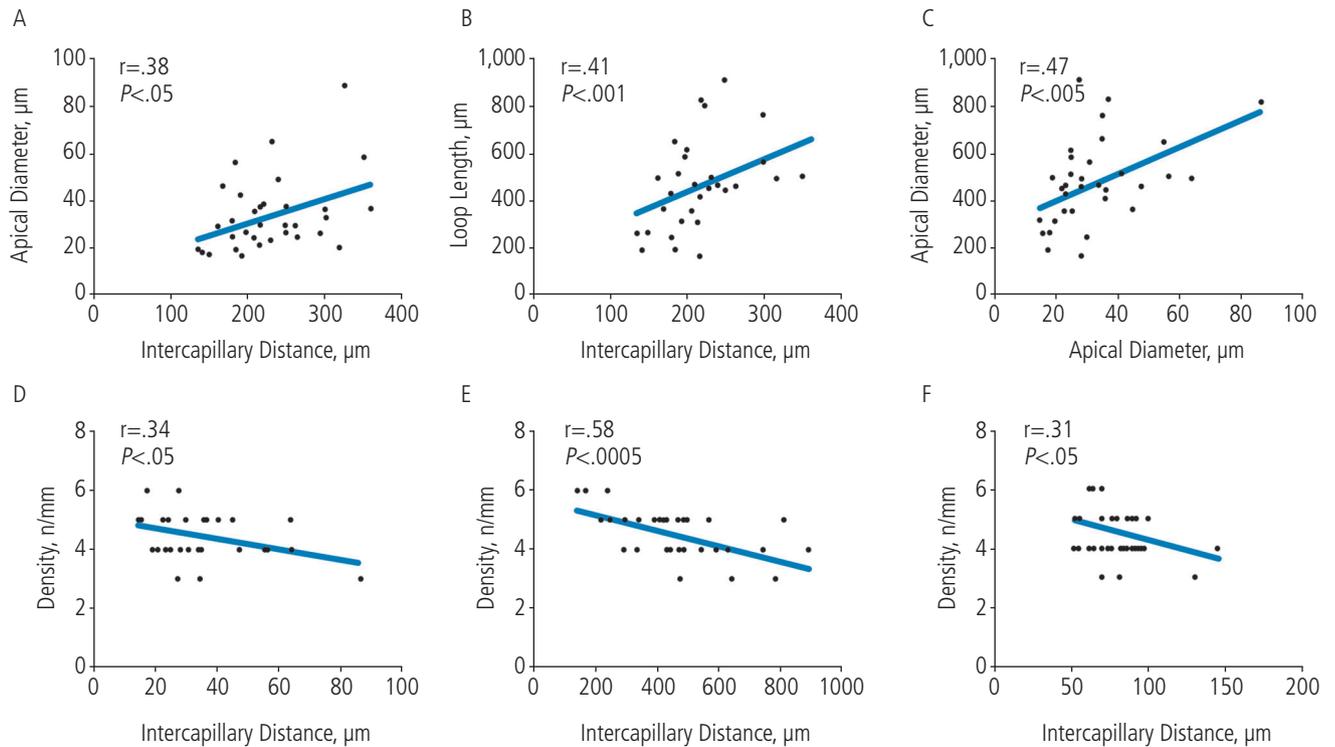
Figure 3. Correlations between 2 capillaroscopic parameters in C1-INH-HAE patients. Apical diameter and intercapillary distance (A), loop length and intercapillary distance (B), loop length and apical diameter (C), density and apical diameter (D), density and loop length (E), and density and external diameter (F) were assessed using Spearman correlation analysis and reported as the correlation coefficient (r).
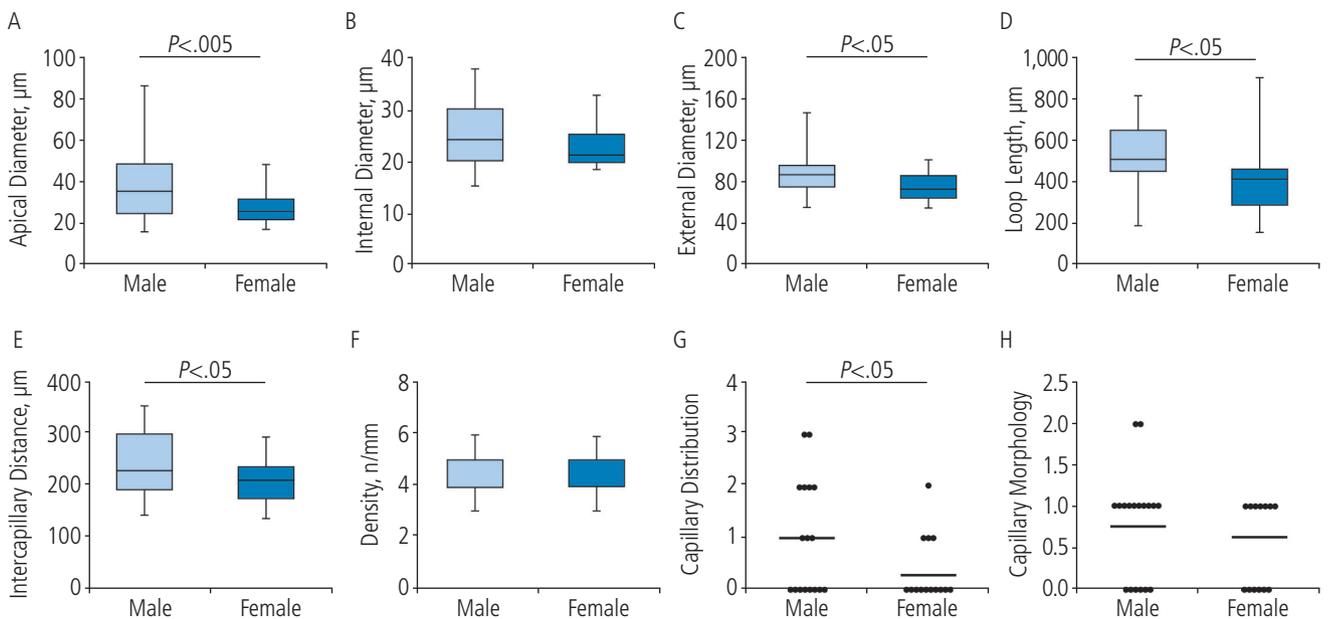

Figure 4. Relationship between capillaroscopic parameters and sex of C1-INH-HAE patients. Apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) were determined in 17 males 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. $P \leq .05$ was considered significant.
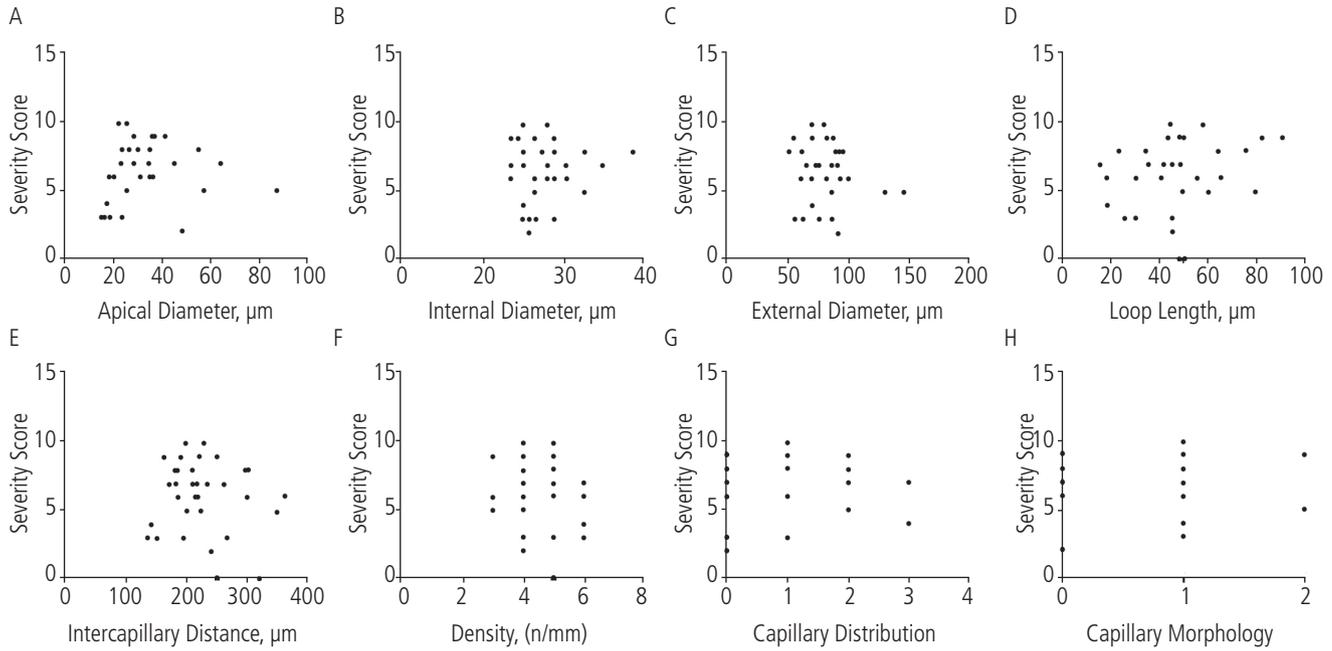
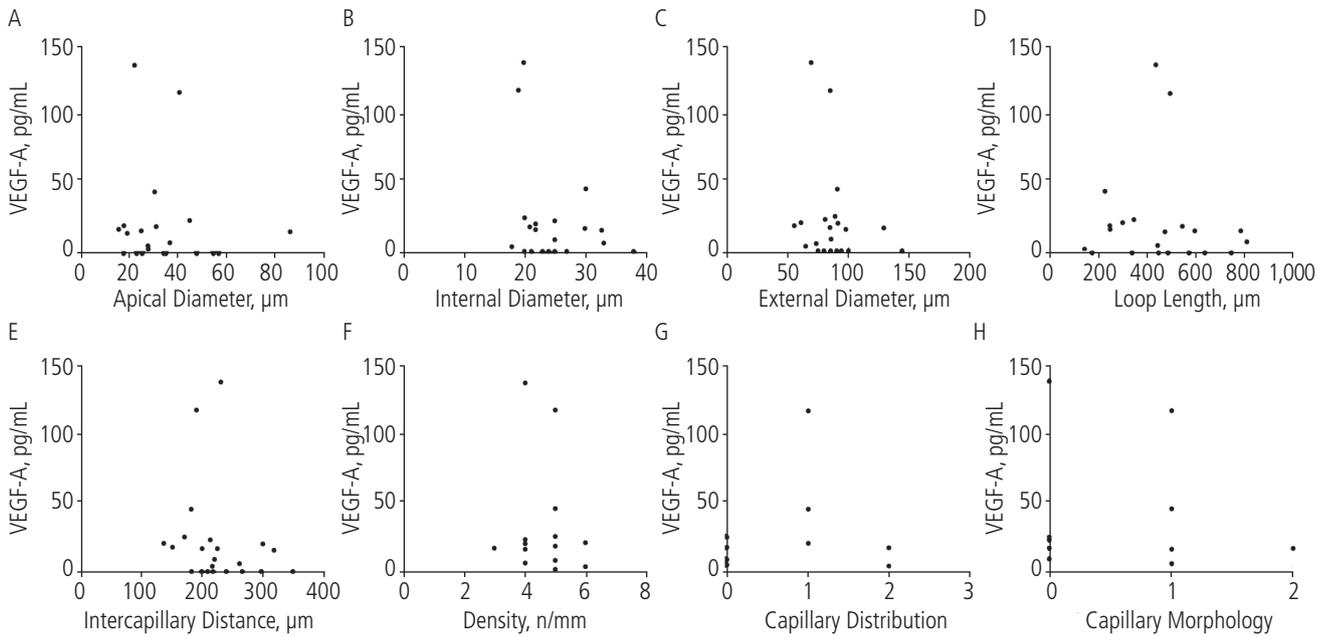**Figure 5.** Correlations between capillaroscopic parameters and disease severity. The correlation between apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) and severity score were assessed using Spearman correlation analysis and reported as the correlation coefficient (r). A *P* value ≤.05 was considered significant.
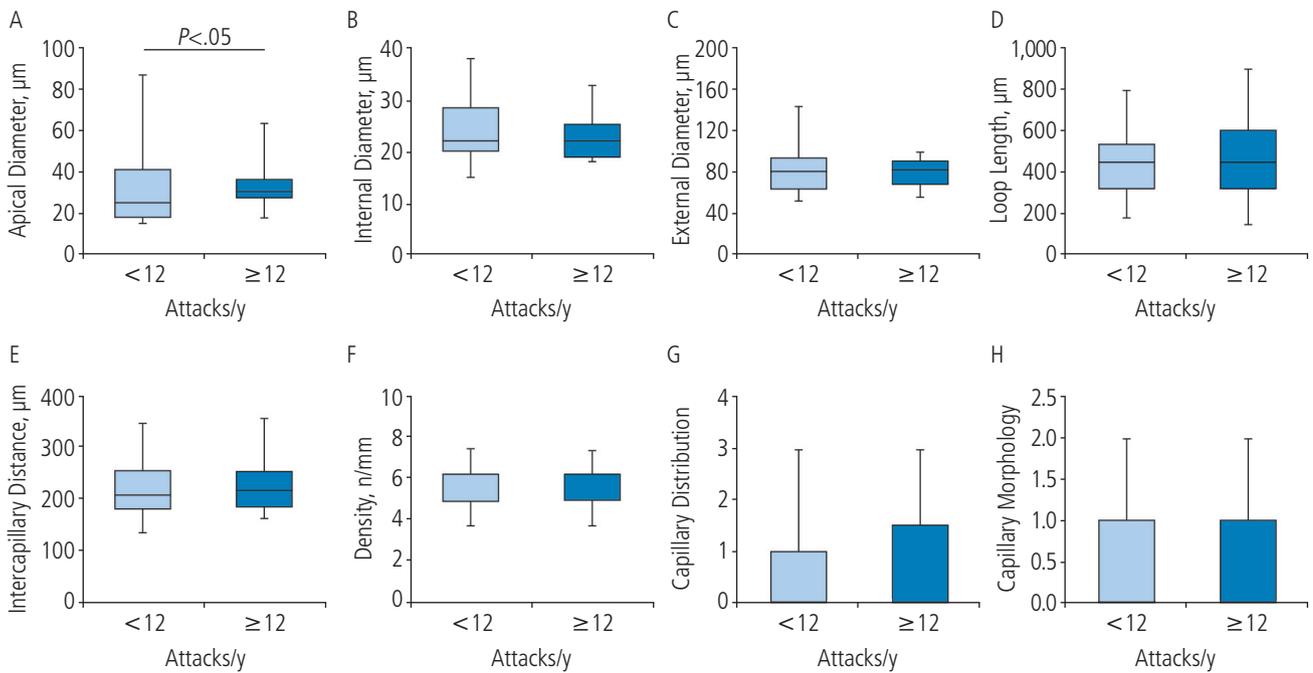
Figure 6. Relationship between capillaroscopic parameters and frequency of attacks in C1-INH-HAE patients. Apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), distribution (G), and morphology (H) were determined in 17 patients with a low frequency of attacks (<12/y) and 17 patients with a high frequency of attacks (≥12/y). 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.
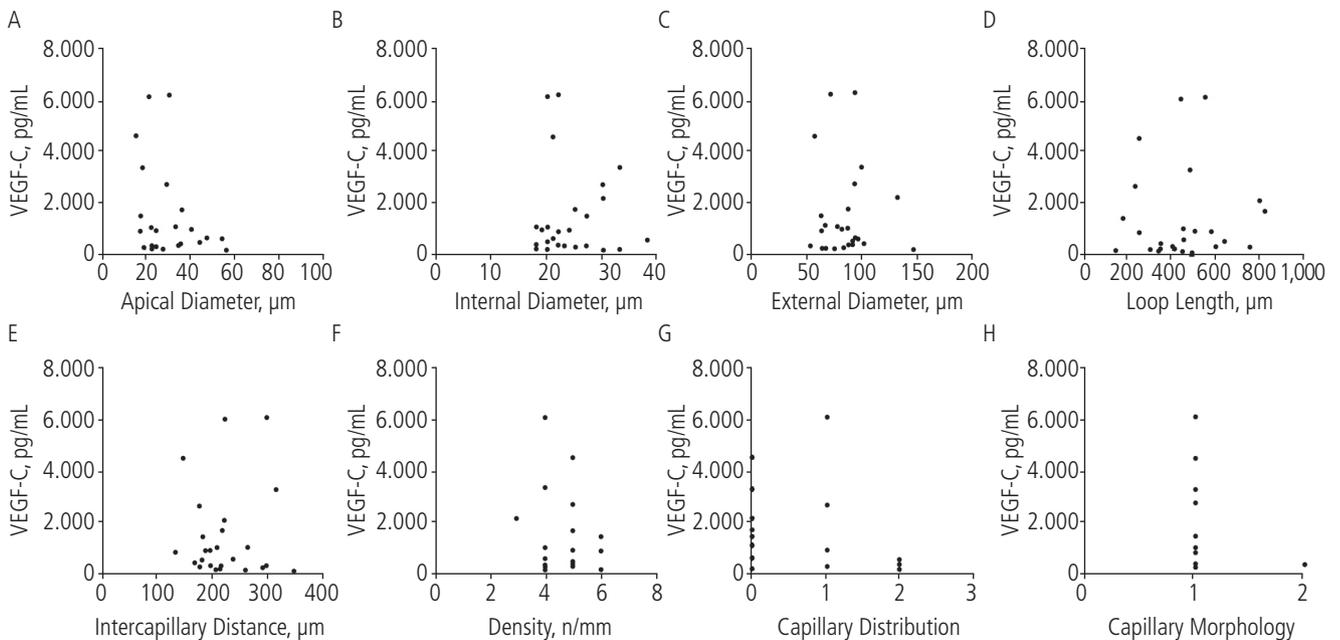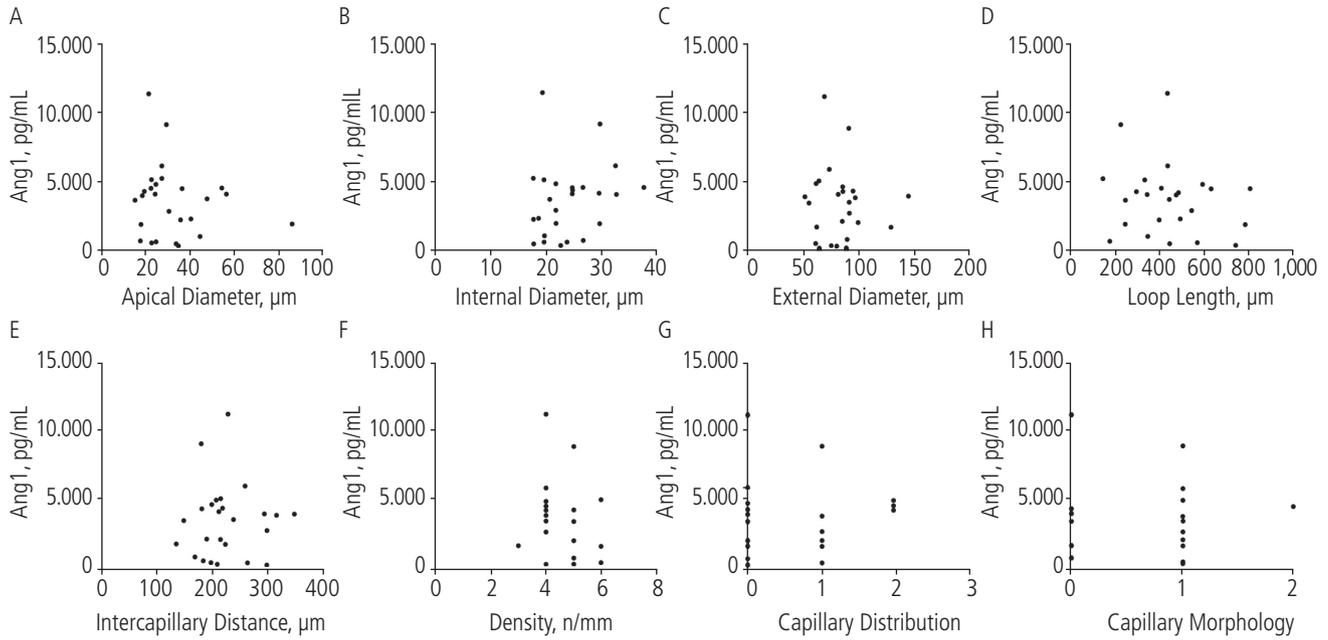
Figure 7. Correlations between capillaroscopic parameters and plasma concentrations of VEGF-A. The correlation between apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) and VEGF-A levels (measured by ELISA) in C1-INH-HAE patients was assessed using Spearman correlation analysis and reported as the correlation coefficient (r). $P \leq .05$ was considered significant.
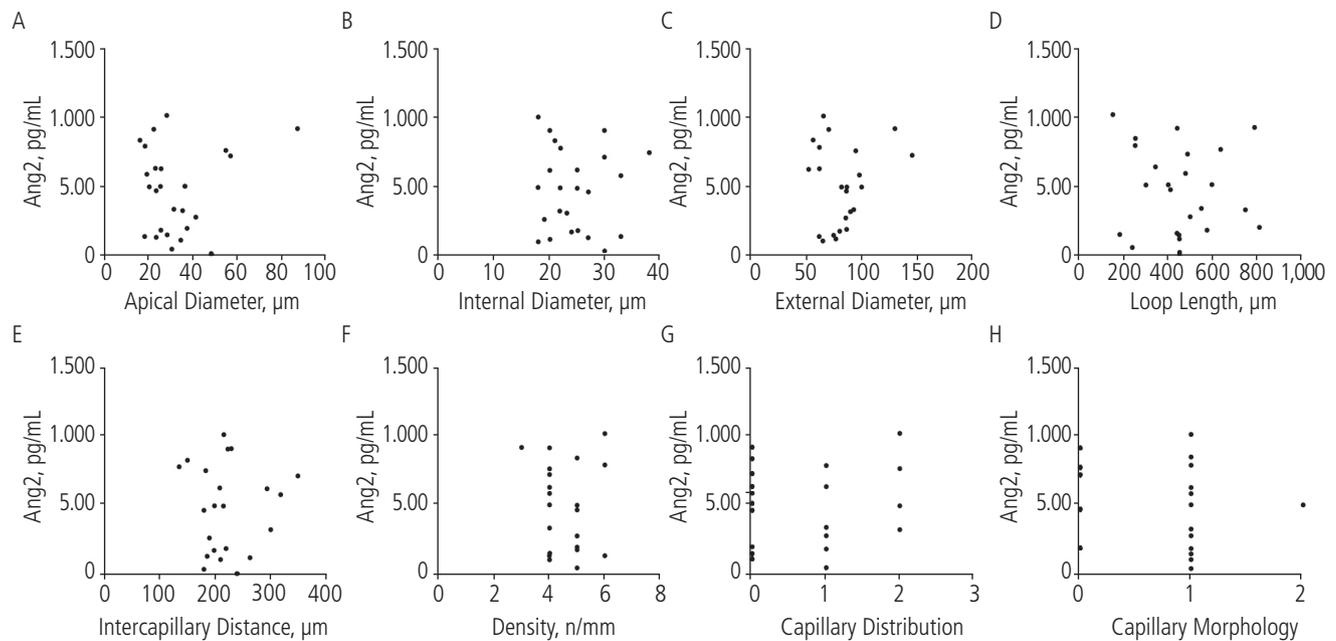

Figure 8. Correlations between capillaroscopic parameters and plasma concentrations of VEGF-C. The correlation between apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) and VEGF-C levels (measured by ELISA) in C1-INH-HAE patients was assessed using a Spearman correlation analysis and reported as the correlation coefficient (r). $P \leq .05$ 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 was assessed using Spearman correlation analysis and reported as the correlation coefficient (r). $P \leq .05$ 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 was assessed using Spearman correlation analysis and reported as the correlation coefficient (r). $P \leq .05$ was considered significant.
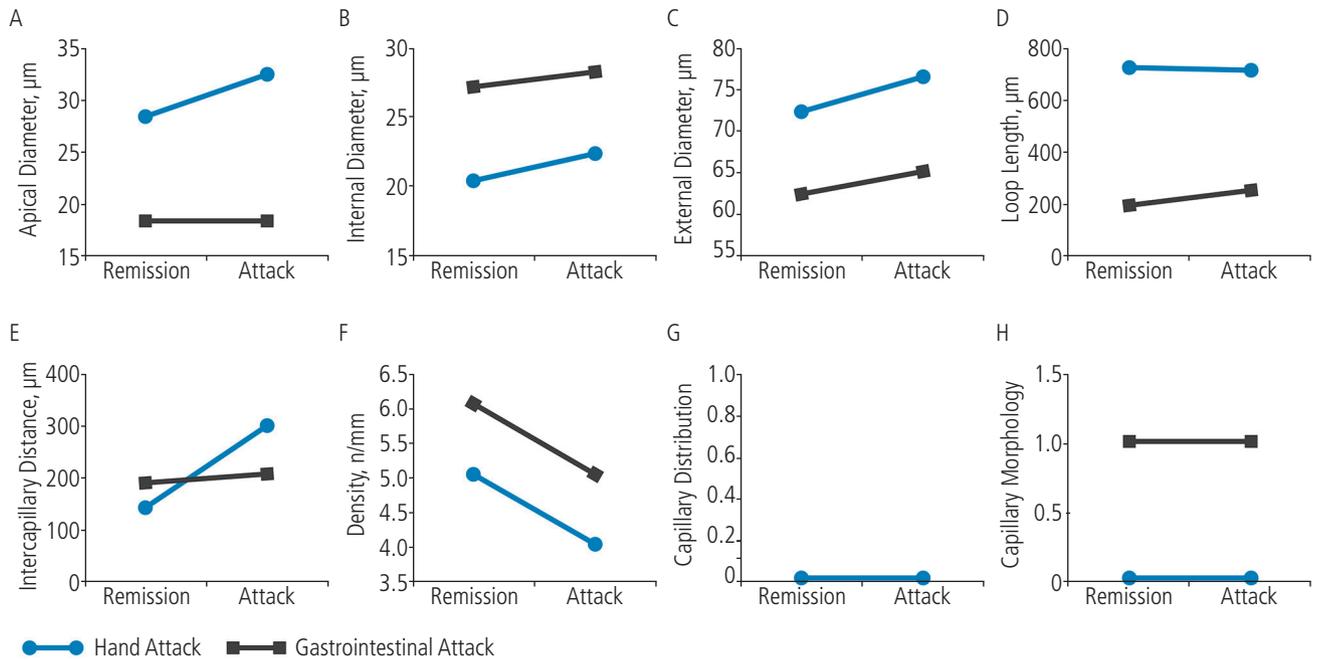
Figure 11. Capillaroscopic parameters in remission and attack periods in C1-INH-HAE patients.
Apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), capillary distribution (G), and capillary morphology (H) were determined in 2 patients with C1-INH-HAE during the symptom-free period and during an angioedema attack. Squares refer to a gastrointestinal attack, while circles indicate a hand attack.
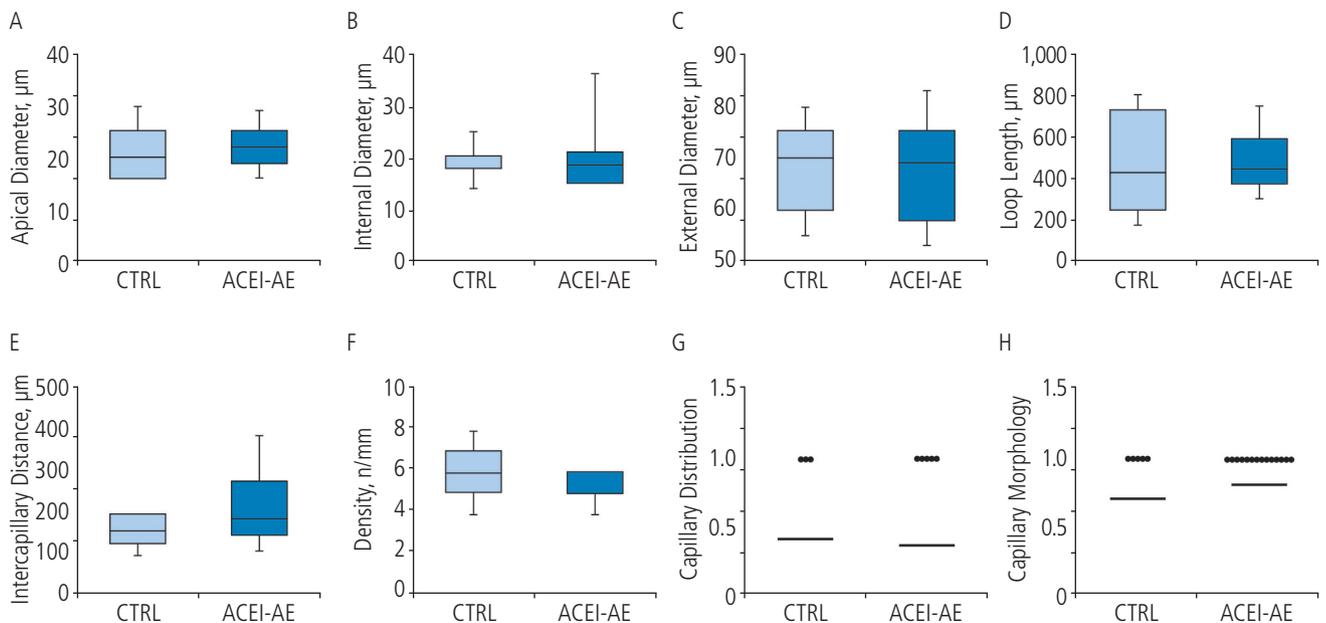

Figure 12. Capillaroscopic parameters in ACEI-AAE patients and healthy controls. Apical diameter (A), internal diameter (B), external diameter (C), loop length (D), intercapillary distance (E), density (F), distribution (G), and morphology (H) were measured in 16 ACEI-AAE patients during the symptom-free period and in 7 hypertensive controls. Horizontal bars depict the median value (A-H), boxes the 25th and 75th percentiles, and whiskers the 5th and 95th percentiles (A-F).

Capillaroscopy Parameters in ACEI-AAE Patients and Controls

In a final series of experiments, we investigated whether capillaroscopy abnormalities were the same in an acquired type of BK-AE and in C1-INH-HAE patients.

Sixteen patients affected by ACEI-AAE (median age, 72.5 [range, 46-82] years; 7 female patients) were also recruited, as were 7 hypertensive participants with similar comorbidities but not affected by ACEI-AAE (median age, 65 [range, 42-78] years; 1 female participant).

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

Discussion

The present study is the first to evaluate structural capillary alterations in patients with angioedema.

The capillaries of patients affected by C1-INH-HAE are characterized by significant quantitative and qualitative morphological alterations, ie, increased diameter (apical, internal, and external), reduced capillary density, and increased prevalence of irregular capillary distribution and tortuous morphology.

Patients with C1-INH-HAE were also characterized by deranged capillary structure, namely, capillaries had become progressively larger and less numerous.

It remains unknown whether these alterations trigger attacks or if, conversely, they 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" that predisposes patients to angioedema attacks. Consequently, the endothelium may become a new therapeutic target.

NVC was performed during acute attacks in only 2 patients, who showed no significant changes compared with baseline. While it is clearly not possible to draw conclusions from such a small sample, it is important to remember that performing NVC is problematic during acute attacks. Therefore, our preliminary data suggest that such alterations are constitutive characteristics of C1-INH-HAE that are not associated with the acute phase. However, a larger sample would clearly be necessary to confirm this hypothesis.

Some published studies report results regarding the presence of endothelial dysfunction in C1-INH-HAE patients [12,13].

In a comparison between 128 patients with C1-INH-HAE and 68 healthy controls, we previously hypothesized that patients with C1-INH-HAE are characterized by "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) [5]. This hypothesis was further corroborated by the higher plasma levels of VEGF-A, VEGF-C, and Ang2 detected in patients with ≥12 attacks per year.

Even though capillaroscopy abnormalities were not correlated with plasma levels of VEGF-A, VEGF-C, Ang1, and Ang2 in the present study, the differences observed led to the same conclusion about the presence of vascular preconditioning, as all except one of the parameters analyzed (ie, loop length) differed significantly with respect to controls. Furthermore, the alterations in NVC appear to be related to the frequency of the attack, since apical diameter was greater in C1-INH-HAE patients with ≥12 attacks per year than in those with <12 attacks per year. Given the types of procedures used, it may be necessary to evaluate the histological aspects of the endothelium rather than serum-related factors. We hypothesize that future studies with larger sample sizes will report significant correlations between angiogenic and lymphangiogenic mediators and morphologic vascular changes.

Endothelial dysfunction in hereditary angioedema (due both to C1-INH deficiency and to factor XII deficiency) was also found by Firinu et al [12], who detected a lower reactive hyperemia index with noninvasive finger plethysmography in the fingertips of 24 HAE patients compared with 24 age- and sex-matched healthy controls. HAE patients also had higher plasma levels of asymmetric dimethylarginine. However, these alterations were not associated with disease severity.

Nebenführer et al [14], on the other hand, excluded the presence of endothelial dysfunction in a study involving 33 C1-INH-HAE patients and 30 healthy controls using flow-mediated dilation measured at the brachial artery. The contrasting conclusions with respect to the study of Bassareo et al [15] may arise from the different measurement techniques used and the heterogeneity of the patient populations recruited.

Finally, NVC also revealed many tortuous loops in capillaries in a family affected by unknown hereditary angioedema [16].

In our study, neither qualitative abnormalities (irregular distribution and presence of tortuous capillaries) nor quantitative abnormalities were detected in patients affected by another form of BK-AE, namely, ACEI-AAE. These findings may support the hypothesis that capillary alterations are a feature of hereditary angioedema.

Apical diameter may be a predictor of severity, as C1-INH-HAE patients with ≥12 attacks per year, ie, the most severe forms of angioedema, had a significantly greater apical diameter than those with <12 attacks per year.

A partial explanation may be found in the 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 more severe abnormalities.

Capillary abnormalities in patients with C1-INH-HAE differed according to sex, although their significance has yet to be understood. In contrast, the only sex-related

significant alteration among healthy individuals was a greater intercapillary distance in females. Piotto et al [17], who performed NVC in 100 healthy individuals aged 5 to 18 years, reported that a greater intercapillary distance for girls than boys (P=.011) was also the only significant alteration.

Our findings for healthy controls may improve recording of normal capillaroscopic parameters, since such data are scarce in the literature, and are consistent with those reported for the largest sample of healthy individuals [18].

NVC seems to be the most suitable method for analyzing the endothelium in angioedema patients: it is simple to perform, noninvasive, repeatable, inexpensive, and highly sensitive. To our knowledge, this is the first study to use NVC in hereditary angioedema. We based our protocol and measurement of capillaroscopy parameters on data in the literature in the field of rheumatology, where the technique is widely used and considered a useful prognostic and diagnostic tool [19] that is highly reliable, regardless of clinicians' experience [20]. The nail bed is easily analyzed because the probe and the capillary vessels are parallel to the cutaneous surface at this site [7].

Our study is subject to a series of limitations. First, the sample size was low; this is a common issue in rare diseases. Second, even though most characteristics of patients and healthy controls were similar, some were not (ie, sex in the comparison between ACEI-AAE patients and controls). However, we do not think these discrepancies affect the reliability of our results. Third, parameters were measured during the attack in only 2 patients, thereby making the resulting measures less reliable. Given the unpredictability of onset of an attack, it is not possible to plan such a procedure in the acute phase of this condition.

In conclusion, our data suggest that C1-INH-HAE patients experience important structural alterations of the capillaries, thus confirming the presence of endothelial dysfunction in 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.

While this pilot study, which was performed in a small sample, has limited clinical value, it may pave the way for larger studies that will increase our knowledge of the underlying mechanisms of C1-INH-HAE and enable us to identify possible therapeutic targets.

Acknowledgments

Previous presentation

The main results of this study were presented as a 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 immunomediated 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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