

Angiotensin-Converting Enzyme Inhibitor–Associated Angioedema: From Bed to Bench

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J Investig Allergol Clin Immunol 2020; Vol. 30(4): 272-280

doi: 10.18176/jiaci.0458

■ Abstract

Background: Angiotensin-converting enzyme inhibitor–associated angioedema (ACEI-AAE) affects 0.1%-0.7% of patients treated with ACEIs. While previous research suggests that angioedema attacks result from increased vascular permeability, the pathogenesis is not completely understood.

Objective: This study aimed to describe the clinical, genetic, and laboratory parameters of ACEI-AAE patients and to investigate the role of vascular endothelial growth factors A and C (VEGF-A and VEGF-C), angiotensins 1 and 2 (Ang1/Ang2), and secretory phospholipase A₂ (sPLA₂) in the pathogenesis of ACEI-AAE.

Methods: The clinical and laboratory data of ACEI-AAE patients were collected from 2 angioedema reference centers. Healthy volunteers and ACEI-treated patients without angioedema were enrolled to compare laboratory parameters. Genetic analyses to detect mutations in the genes *SERPING1*, *ANGPT1*, *PLG*, and *F12* were performed in a subset of patients.

Results: A total of 51 patients (57% male) were diagnosed with ACEI-AAE. The average time to onset of symptoms from the start of ACEI therapy was 3 years (range, 30 days-20 years). The most commonly affected sites were the lips (74.5%), tongue (51.9%), and face (41.2%). Switching from ACEIs to sartans was not associated with an increased risk of angioedema in patients with a history of ACEI-AAE. VEGF-A, VEGF-C, and sPLA₂ plasma levels were higher in ACEI-AAE patients than in the controls. Ang1/2 concentrations remained unchanged. No mutations were detected in the genes analyzed.

Conclusions: Our data suggest that sartans are a safe therapeutic alternative in ACEI-AAE patients. Increased concentrations of VEGF-A, VEGF-C, and sPLA₂ in ACEI-AAE patients suggest a possible role of these mediators in the pathogenesis of ACEI-AAE.

Key words: Angiotensin-converting enzyme inhibitor. Angioedema. Vascular endothelial growth factor C. Vascular endothelial growth factor A. Phospholipases A₂. Genetic analysis. C1-inhibitor. Biomarkers.

■ Resumen

Antecedentes: El angioedema asociado al consumo de inhibidores de la enzima convertidora de angiotensina (IECA-AAE) ocurre en el 0,1%-0,7% de los pacientes tratados con IECA. Aunque se sugiere que los ataques de angioedema son el resultado de una mayor permeabilidad vascular, la patogénesis de este proceso no está plenamente esclarecida.

Objetivo: En este trabajo se estudiaron los parámetros clínicos, genéticos y de laboratorio de pacientes con IECA-AAE, así como el papel de los factores de crecimiento endotelial vascular A y C (VEGF-A y VEGF-C), las angiopoyetinas 1 y 2 (Ang1/Ang2) y la fosfolipasa secretora A₂ (sPLA₂).

Métodos: Se recogieron datos clínicos y de laboratorio de pacientes con IECA-AAE procedentes de dos centros de referencia en angioedema. Se utilizaron pacientes control, que incluyeron a voluntarios sanos y a pacientes tratados con IECA sin angioedema, para comparar las concentraciones de los parámetros de laboratorio. Finalmente, se realizó un análisis genético en un subconjunto de pacientes para detectar mutaciones en los genes *SERPING1*, *ANGPT1*, *PLG* y *F12*.

Resultados: Se diagnosticaron a 51 pacientes (57% hombres) con IECA-AAE. El tiempo promedio para el inicio de los síntomas desde el comienzo del tratamiento con IECA fue de 3 años (rango de 30 días a 20 años). Los lugares más comúnmente afectados fueron: labios (74,5%), lengua (51,9%) y cara (41,2%). El cambio de IECA a ARA-II no se asoció con un mayor riesgo de angioedema en pacientes con antecedentes de IECA-AAE. Los niveles plasmáticos de VEGF-A, VEGF-C y sPLA₂ fueron más altos en pacientes con IECA-AAE que en los controles. No se detectaron cambios en las concentraciones de Ang1/Ang2, ni se detectaron mutaciones en los genes analizados.

Conclusiones: Nuestros datos sugieren que los ARA-II pueden ser una alternativa terapéutica segura en pacientes con IECA-AAE. El aumento de las concentraciones de VEGF-A, VEGF-C y sPLA₂ en pacientes con ACEI-AAE sugiere un posible papel de estos mediadores en la patogénesis de esta enfermedad.

Palabras clave: Inhibidor de la enzima convertidora de angiotensina. Angioedema. Factor de crecimiento endotelial vascular C. Factor de crecimiento endotelial vascular A. Fosfolipasa A₂. Análisis genético. Inhibidor de C1. Biomarcadores.

Introduction

Angioedema is localized and self-limiting edema involving the subcutaneous tissues and submucosa that may be an expression of either allergic or nonallergic diseases. Angiotensin-converting enzyme inhibitor-associated angioedema (ACEI-AAE), a form of acquired angioedema without wheals [1,2], is a rare adverse effect of ACEIs that affects 0.1%-0.7% of patients [3].

The pathogenesis of ACEI-AAE is not completely understood. Previous studies have demonstrated that the pharmacological inhibition of ACE leads to reduced catabolism of vasoactive mediators (eg, bradykinin, substance P), which may result in their accumulation and a consequent increase in vascular permeability. However, the pathogenesis of ACEI-AAE remains uncertain, as only a small percentage of ACEI-treated patients experience angioedema, and the time to onset of symptoms varies considerably. Therefore, predisposing factors may exist, and other mediators might contribute to the pathogenesis and/or regulation of vascular permeability in ACEI-AAE. Our group recently demonstrated an increased plasma concentration of vascular endothelial growth factors (VEGFs) and angiopoietins (Angs) [4] and increased activity of secretory phospholipase A₂ (sPLA₂) [5] in patients with hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE). Thus, evaluating the clinical and laboratory characteristics of vascular permeability in patients with other forms of bradykinin-mediated angioedema, such as ACEI-AAE, may elucidate the pathogenesis of the condition and classification of patients treated with ACEIs who are potentially at risk of developing this adverse event.

Recently, Firinu et al [6] demonstrated the importance of conducting genetic investigations in patients with different forms of idiopathic angioedema for accurate diagnostic work-ups. When clinicians encounter a recurrent form of angioedema with normal C1-INH levels and no specific diagnostic biomarkers, distinguishing between hereditary and acquired angioedema is difficult. Family history and age at onset may occasionally be informative because there are forms of angioedema where family history is negative, age of onset is advanced, and ACEI treatment triggers activation of the carrier state [7], leading to a misdiagnosis of ACEI-AAE. These issues may arise, for example, in the case of *F12* mutations with incomplete penetrance, which are carried by 90% of asymptomatic angioedema patients [6,8]. The absence of a family history has been observed in other recently discovered hereditary forms associated with mutations in the *PLG* gene [9-11] and *ANGPT1* gene [12], in which patients were initially

diagnosed with acquired idiopathic angioedema that was later shown to be hereditary.

We assessed the clinical and laboratory features of a cohort of white ACEI-AAE patients. The investigations included the possible risk and/or protective factors for development of ACEI-AAE, possible correlations between the clinical features and laboratory parameters (levels of VEGFs and Angs and sPLA₂ activity) of ACEI-AAE patients, risk of angiotensin II receptor blocker (ARB)-induced angioedema in patients with a history of ACEI-AAE, and the utility of extensive genetic screening to identify rare hereditary forms of angioedema.

Methods

Participants

The study population was a cohort of white patients with ACEI-AAE from 2 angioedema reference centers (Center for the Diagnosis and Therapy of Angioedema, University Federico II, Naples, Italy; Division of Clinical Immunology, Allergy and Rheumatology, University Hospital Dubrava, Zagreb, Croatia). Healthy volunteers and ACEI-treated individuals without angioedema were enrolled as controls for the evaluation of vascular permeability factors (VEGFs, sPLA₂, and Angs). Genetic analyses to detect mutations in the genes *SERPING1*, *ANGPT1*, *PLG*, and *F12* were performed in a subset of patients.

Diagnoses were based on clinical symptoms, plasma levels, activity of C1-INH, and C4 levels. Patient data were collected from medical records.

The study was approved by the Ethics Committee (protocol number 216/16) and conducted in accordance with the Declaration of Helsinki.

Blood Sampling

Blood was collected during routine diagnostic procedures, and all patients gave their oral informed consent for the remaining plasma to be used for research purposes. The plasma samples were labeled with codes that were recorded on a data sheet. The control patients had been referred for a routine medical check-up and volunteered for the study by giving their informed consent. Technicians performing the assays were blinded to the patients' history. Blood samples from all patients were obtained at least 8 days after an angioedema attack (remission sample). The samples were collected via clean venipuncture with minimal stasis using 3.2% sodium citrate. After centrifugation at 2000g for 20 minutes at room

temperature, the plasma was divided into aliquots and stored at -80°C until testing.

Complement System

The activity of C1-INH was measured using a commercially available kit containing a specific chromogenic substrate (Technoclone GmbH).

Determination of VEGFs and Angs

Plasma levels of angiogenic and lymphangiogenic mediators were measured using commercially available ELISA kits for VEGF-A, VEGF-C, Ang1, and Ang2 (R&D Systems) according to the manufacturer's instructions. The sensitivity of ELISA was 31.1-2000 pg/mL for VEGF-A, 62-4000 pg/mL for VEGF-C, 156.25-10 000 pg/mL for Ang1, and 31.1-4000 pg/mL for Ang2.

Testing of sPLA₂ Function

The activity of sPLA₂ in the plasma of ACEI-AAE patients, healthy controls and patients treated with ACEIs without angioedema was measured using the EnzChek phospholipase A₂ assay (Life Technologies). Briefly, an sPLA₂ substrate cocktail consisting of 7-hydroxycoumarinyl-arachidonate (0.3 mM), 7-hydroxycoumarinyl-linolenate (0.3 mM), hydroxycoumarinyl-6 heptanoate (0.3 mM), dioleoylphosphatidylcholine (10 mM), and dioleoylphosphatidylglycerol (10 mM) was prepared in ethanol. Liposomes were formed by gradually adding 77 μL of substrate/lipid cocktail to 10 mL of PLA2 buffer (50 mM Tris-HCl, 100 mM NaCl, and 1 mM CaCl₂) while stirring rapidly for 1 minute with a magnetic stirrer. Fluorescence was measured (excitation at 360 nm and emission at 460 nm), and the specific activity (relative fluorescent units/mL) was calculated for each

sample. Fifty-microliter aliquots of plasma from ACEI-AAE patients, patients treated with ACEIs without angioedema, and healthy controls were added to 96-well plates, and sPLA₂ activity in the samples was measured by adding 50 μL of substrate cocktail.

Genetic Screening

Direct DNA sequencing was conducted using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequences obtained were compared with consensus sequences from specific databases (Ensembl 2011).

Statistical Analysis

Collected data were processed using statistical software (GraphPadPrism 5, GraphPad Software) and tested for normality using the D'Agostino-Pearson normality test. Data with a normal distribution determined at a significance level of .05 were analyzed using parametric tests. Nonparametric tests were used for nonnormally distributed data. Two-tailed *t* tests were performed for independent samples, and 2-tailed Mann-Whitney tests were performed as indicated in the figure legends. The correlation between 2 variables was assessed using the Pearson or Spearman test and reported as a correlation coefficient (*r*). A *P* value $\leq .05$ was considered statistically significant.

Results

Clinical Characteristics

A total of 51 white patients with ACEI-AAE were included in this study. The controls comprised 86 healthy participants and 20 ACEI-treated participants without angioedema. The Table summarizes the clinical features of all the participants.

Table 1. Clinical Characteristics

	ACEI-AAE Patients (n=51)	Healthy Controls (n=86)	ACEI-Treated Without AE (n=20)
Male:Female, No. (%)	29:22 (56.8:43.2)	46:40 (53.5:46.5)	11:9 (55:45)
Median (IQR) age at diagnosis, y	64 (42-90)	NA	NA
Median (IQR) age at onset of symptoms, y	63 (42-80)	NA	NA
Hospitalization, No. (%)	32 (62.7)	NA	NA
Associated symptoms, No. (%)	12 (23.5)	NA	NA
– Itching, No. (%)	– 9 (75)		
– Rash, No. (%)	– 3 (25)		
Comorbidities		NR	
– Diabetes, No. (%)	– 7 (14)		– 9 (56.2) ^a
– Atopy, No. (%)	– 12 (23.5)		– 3 (21.4) ^b
Allergies to other drugs, No. (%)	8 (15.7)	NR	3 (21.4) ^b
Smoking, No. (%)	23 (45)	NR	6 (30)
Family history for ACEI-AAE, No. (%)	3 (5.9)	NA	1 (5)

Abbreviations: ACEI-AAE, angiotensin-converting enzyme inhibitor-associated angioedema; AE, angioedema; NA, not applicable; NR, not reported.

^aData reported for 16 patients.

^bData reported for 14 patients.

The median interval between the start of ACEI therapy and the onset of angioedema was 3 years (range, 3 days to 20 years). The median duration of ACEI therapy was 4 years (range, 3 days to 20 years). Most patients (39.2%) experienced the first attack of acute angioedema between the first and fifth year after beginning ACEI therapy, 35.3% after 5 years of therapy, and 25.5% within a year of beginning ACEI therapy.

Among the ACEI-AAE patients, ACEI prescriptions included ramipril (27.5%), enalapril (23.5%), lisinopril (17.6%), zofenopril (13.7%), perindopril (11.8%), fosinopril (3.9%), and delapril (2%). In ACEI-treated patients without angioedema, ACEI prescriptions included ramipril (70%), lisinopril (15%), enalapril (10%), and zofenopril (5%).

After initiation of ACEI therapy, the median number of total angioedema attacks per patient was 3 (range, 1-180). It was not possible to calculate the mean frequency of angioedema attacks per year, as 31.4% of patients discontinued ACEI after the first or second episode.

Attack frequency by site was as follows: lips, 74.5%; tongue, 51.9%; face, 41.2%; upper airways, 29.5%; skin, 17.6%; genitals, 5.9%; and abdomen, 3.9%. Values are expressed as noncumulative percentages, that is, patients may have exhibited attacks involving multiple locations simultaneously.

The average duration of an angioedema attack was 24 hours (range, 3-168 hours). The attacks lasted ≥ 24 hours in 56.8% of patients, 12-23 hours in 35.4%, and less than 12 hours in 7.8% of patients.

After diagnosis, patients were followed for up to 12 years, with a minimum follow-up time of 6 months. ACEIs were suspended in 98% of patients, and only 1 patient (2%)

continued ACEIs despite persistent angioedema attacks. The control group with ACEI-treated patients without angioedema were treated with ACEIs for a minimum of 6 months to 20 years.

After discontinuation of ACEI therapy, 68% (34 patients) no longer experienced angioedema. Among these patients, 41.2% took ARBs without presenting angioedema. Only 32% (16 patients) continued to have angioedema episodes despite the suspension of ACEIs (Figure 1).

Analysis of antihypertensive replacement therapy revealed that 44% (22/50) switched to ARBs, while 56% of patients (28/50) started other antihypertensive drugs or discontinued antihypertensive therapy (36% switched to calcium antagonists, 14% to no therapy, and 8% to β -blockers, α -blockers, or diuretics). We found that 9/22 patients developed angioedema during treatment with ARBs, although for 2 patients, angioedema was not related to ARBs (1 with spontaneous resolution despite continuation of ARBs; 1 with persistent angioedema after discontinuation of ARBs). Ultimately, ARB-induced angioedema was confirmed in 31.8% of patients (7/22). The prevalence of angioedema in patients treated with other antihypertensive drugs was 28.6% (8/28).

Among the patients with persistent attacks, these recurred in 50% of patients treated with ramipril, perindopril, or fosinopril, 28.5% of patients treated with zofenopril, 11% of patients treated with lisinopril, and 8.3% of patients treated with enalapril.

Prior to the diagnosis of ACEI-AAE, all patients received corticosteroids and/or antihistamines during acute attacks, with no improvement in symptoms. Three patients received intramuscular adrenaline as adjunctive therapy but did not

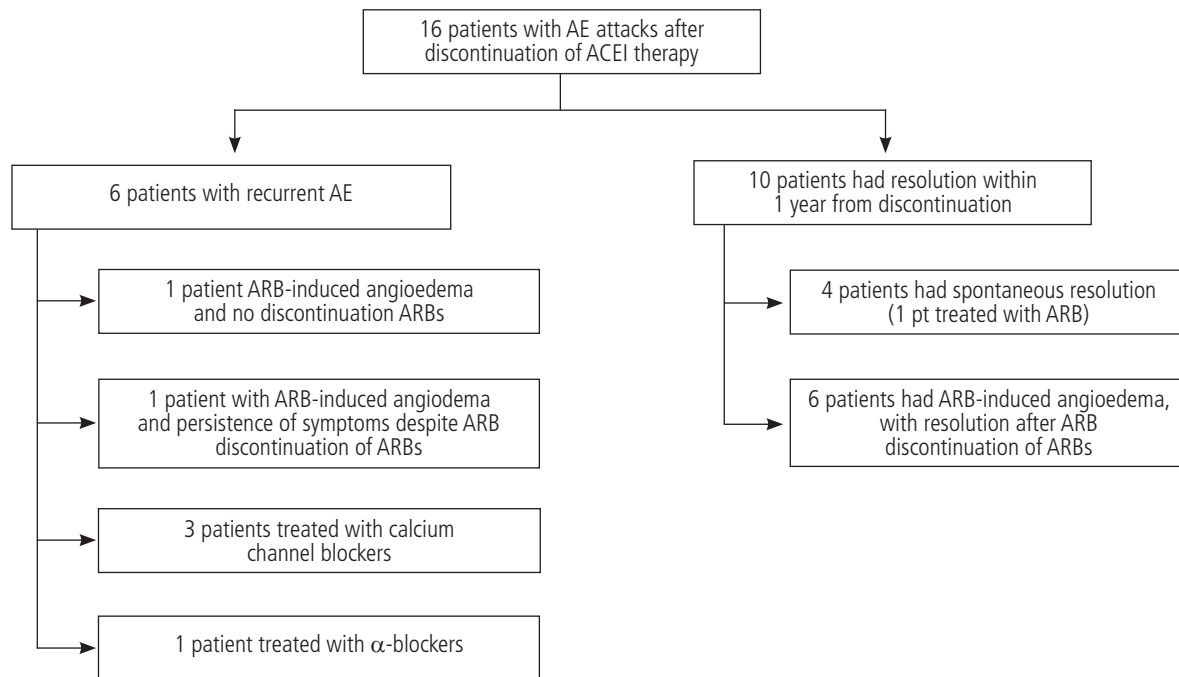


Figure 1. Patients with persistent angioedema attacks after discontinuation of ACEI therapy. AE, angioedema; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

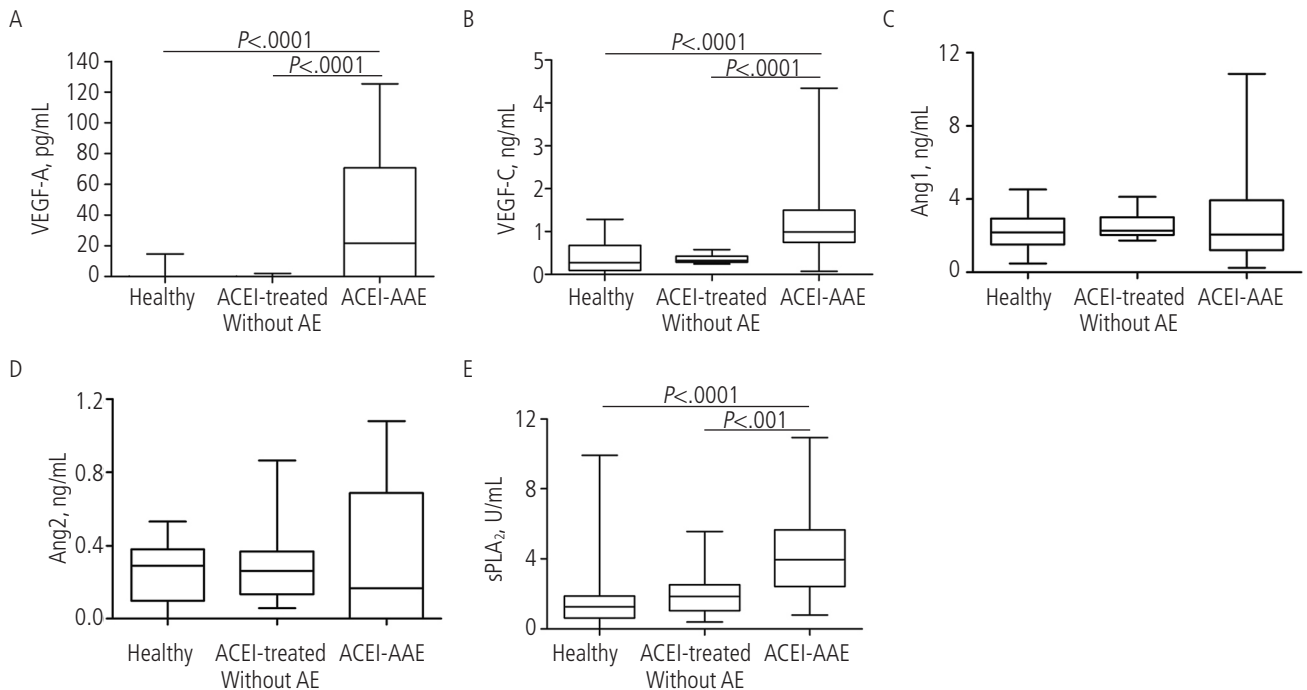


Figure 2. Plasma concentrations of VEGF-A, VEGF-C, Ang1, Ang2, and sPLA₂ in the study population. Plasma VEGF-A (A), VEGF-C (B), Ang1 (C), Ang2 (D) and sPLA₂ (E) in controls (Healthy), ACEI-treated individuals without angioedema (ACEI-treated w/o angioedema), and patients with ACEI-AAE in remission. Data 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. VEGF indicates vascular endothelial growth factor; AE, angioedema; ACEI-AAE, angiotensin-converting enzyme inhibitor-associated angioedema; Ang, angiopoietin; sPLA₂, secretory phospholipases A₂.

report positive effects on the duration of the episodes or adverse effects related to the drug. One patient received icatibant as add-on therapy, with no significant reduction in the duration of the attacks.

Laboratory Parameters

Figures 2A and 2B show that plasma VEGF-A and VEGF-C levels in patients with ACEI-AAE were higher than in the healthy controls and ACEI-treated individuals without angioedema. Median (IQR) VEGF-A values were 21.7 (0-70.9), 0 (0-0), and 0 (0-0) pg/mL, respectively. Median VEGF-C values were 0.9 (0.7-1.5), 0.268 (0.09-0.6), and 0.28 (0.24-0.32) ng/mL (interquartile ranges), respectively.

In contrast, the Ang1 and Ang2 plasma levels of patients with ACEI-AAE were not altered compared with those of the healthy controls or ACEI-treated individuals without angioedema (Figure 2C-2D).

sPLA₂ activity was approximately 3 times higher in patients with ACEI-AAE than in healthy controls or ACEI-treated individuals without angioedema. The median enzymatic activities of the groups were 3.9 (2.4-5.7), 1.27 (0.6-1.8), and 1.86 (1.05-2.5) U/mL, respectively (Figure 2E).

According to our data, some ACEIs led to angioedema more frequently than others. Therefore, we divided the ACEI-AAE patients into groups based on their ACEI therapies and evaluated the concentrations of VEGF-A, concentrations of VEGF-C, and sPLA₂ activity within each group. Patients

treated with enalapril, ramipril, and zofenopril had higher levels of VEGF-A, VEGF-C, and sPLA₂ than the controls (Figure 3).

We also investigated possible correlations between laboratory parameters and clinical features of angioedema attacks. We found no significant relationship between plasma concentrations of VEGF-A and VEGF-C, activity of sPLA₂, and time between the onset of therapy and onset of symptoms. Furthermore, no correlations were observed between the laboratory parameters and age at onset of symptoms, duration of episodes, or number of sites involved.

To determine whether ACEI therapy influenced plasma levels of VEGF-A and VEGF-C and the activity of sPLA₂, we assessed the relationship between the laboratory parameters and the interval between plasma sampling and discontinuation of ACEI therapy. However, the results did not reveal a significant correlation between this clinical feature and variations in the laboratory parameters (Figure 4).

In a further analysis, the patients were divided into 3 groups according to persistence of angioedema attacks after discontinuation of ACEI. The groups included patients whose attacks persisted after discontinuation, patients who experienced attacks within 1 year after discontinuation, and patients whose symptoms resolved following discontinuation (no attacks). We then analyzed the laboratory parameters of the 3 patient groups. Figure 5 shows that the plasma concentrations of VEGF-A and VEGF-C and the activity of sPLA₂ did not vary in the 3 groups analyzed.

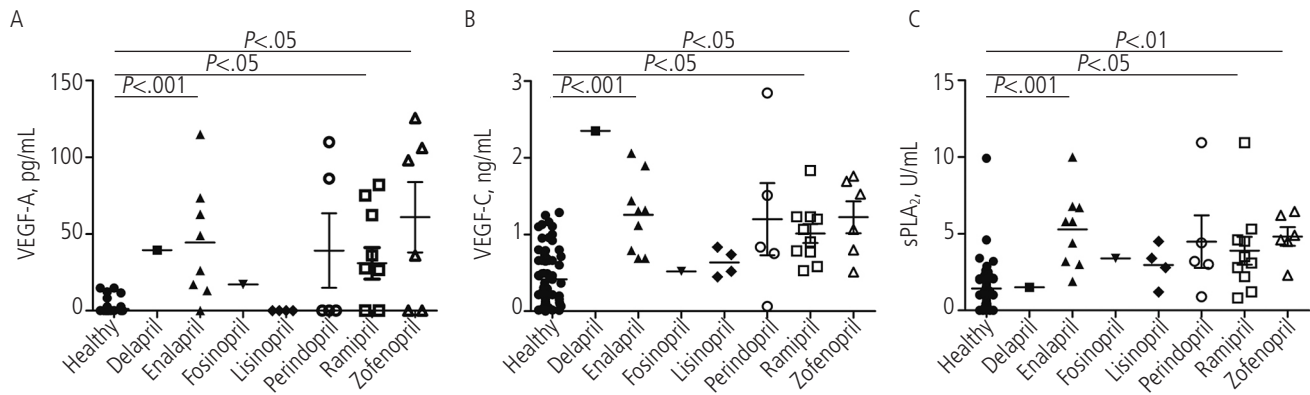


Figure 3. Plasma levels of VEGF-A and VEGF-C and plasma activity of sPLA₂ in healthy subjects and ACEI-AAE patients by type of ACEI. VEGF indicates vascular endothelial growth factor; sPLA₂, secretory phospholipase A₂; ACEI-AAE, angiotensin-converting enzyme inhibitor-associated angioedema; ACEI, angiotensin-converting enzyme inhibitor.

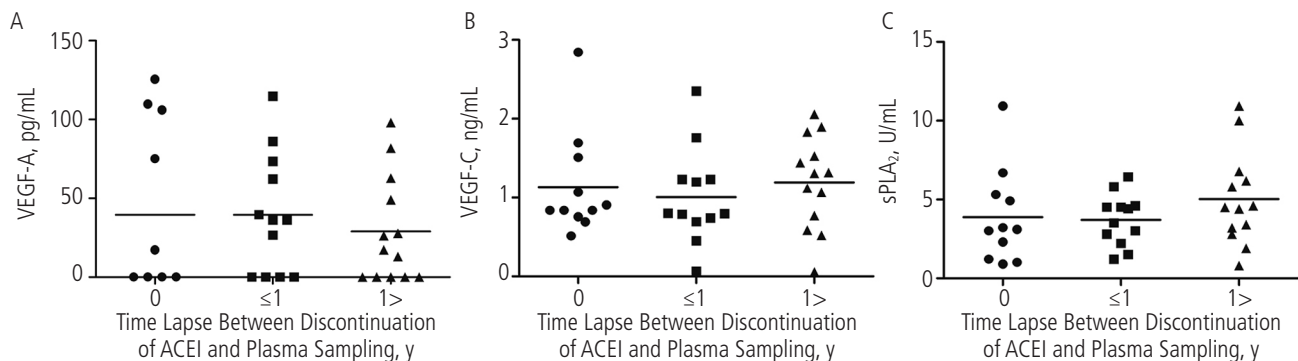


Figure 4. Correlation between plasma levels of VEGF-A, VEGF-C, and sPLA₂ and the time between discontinuation of ACEI and plasma sampling (years) (0, plasma sampling at discontinuation of ACEI; ≤1, plasma sampling within 1 year from discontinuation of ACEI; >1, plasma sampling more than 1 year from discontinuation of ACEI). VEGF indicates vascular endothelial growth factor; sPLA₂, secretory phospholipases A₂.

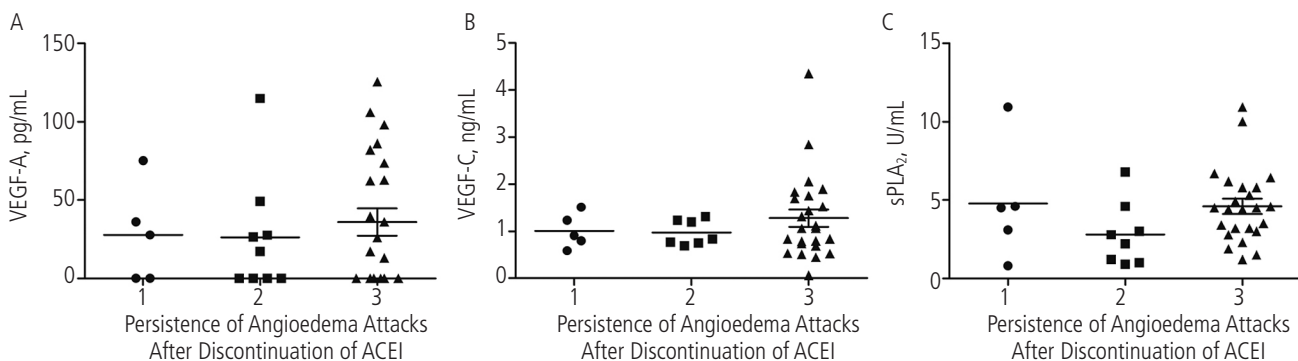


Figure 5. Correlation between the plasma levels of VEGF-A, VEGF-C and sPLA₂ and the persistence of angioedema attacks after discontinuation of ACEI (1, persistence of the attacks; 2, attacks within 1 year after discontinuation of ACEI; 3, no attacks after discontinuation of ACEI). VEGF indicates vascular endothelial growth factor; sPLA₂, secretory phospholipases A₂; ACEI, angiotensin-converting enzyme inhibitor.

Genetic Characteristics of the Population

We performed a genetic analysis of the *SERPING1* gene, *ANGPT1* gene, the ap.Thr309Lys missense mutation in the *F12* gene, and the p.Lys330Glu missense mutation in the *PLG* gene in 33 patients, including 5 patients whose angioedema episodes persisted after discontinuation of ACEIs. No mutations were detected.

Discussion

Angioedema is a rare but potentially life-threatening adverse effect of ACEIs. Diagnosis and treatment of acute angioedema are challenging. Given the vast number of patients who are treated with ACEIs, identifying those potentially at risk of developing ACEI-AAE is highly relevant and complicated.

Here, we report the clinical characteristics of a white patient cohort and highlight correlations that only partially confirm previous data regarding the possible roles of female sex, smoking, and age >65 years as risk factors associated with ACEI-AAE. Our results differ from those reported in the literature in terms of the correlations we found with sex, as most of our patients were male [13-15]. Furthermore, smokers and nonsmokers were almost uniformly distributed in our study (45% vs 55%). A correlation between ACEI-AAE and age was recorded; however, this was not strong, and approximately 50% of our patients were ≥ 65 years of age.

Consistent with our results, diabetes is commonly reported in the literature as a protective factor (14% of patients with diabetes vs 86% without), supporting the role of DPP-4 as a major enzyme in the catabolism of bradykinin [16-18]. A more controversial topic is the correlation between IgE-mediated diseases and ACEI-AAE. While some studies consider a history of atopy and drug allergy to be risk factors for ACEI-AAE [13,14], others do not find a significant relationship between the 2 mechanisms [15]. In our study population, a history of atopy and drug allergy did not appear to be risk factors for ACEI-AAE, as they were only present in a small percentage of patients (23.5% and 15.7%, respectively). However, they were associated with an increased frequency of histamine-mediated symptoms, such as pruritus and rash, during episodes of angioedema. Of the 8 patients with drug allergy, 50% exhibited associated symptoms during angioedema episodes, as if the tendency of these patients to develop histamine-mediated drug reactions conferred susceptibility to the release of histamine during episodes of bradykinin-mediated angioedema. Some authors propose that an initial histamine release might trigger bradykinin-mediated angioedema attacks, although this hypothesis has yet to be supported by sufficient evidence [19,20]. In addition, there is evidence to support the notion that classic antiallergic drugs such as antihistamines and corticosteroids are not effective for treatment of ACEI-AAE, and that while histamine does make a contribution, this is not significant.

The time frame for presentation of ACEI-AAE varies widely. Data from our cohort show that ACEI-AAE may develop at any time after the initiation of ACEI therapy, from 3 days to 20 years after the first administration, which is consistent with trends reported elsewhere [21,22].

The ACEI most correlated with onset of angioedema in our cohort was ramipril (27.5%), followed by enalapril (23.5%) and lisinopril (17.6%). Although these drugs, especially enalapril, are among the most prescribed in their class in Italy [23], we cannot exclude a potentially higher risk of developing ACEI-AAE in patients taking these drugs than in patients taking other ACEIs. The increased risk may be partially explained by the long half-life of enalapril (11 hours), ramipril (15 hours), and lisinopril (12 hours), along with the greater potency—expressed as the IC_{50} (ie, the concentration required to inhibit 50% of enzymatic activity)—of these ACEIs than of others [24]. Montoro et al [25] showed that enalapril was associated with a greater risk of developing ACEI-AAE than other ACEIs. A greater percentage of ACEI-AAE has been recorded in patients treated with lisinopril [22,26,27], possibly

because lisinopril was the most prescribed drug in the study population, although lisinopril is also part of the triad of ACEIs with a longer half-life and greater potency.

After discontinuation of ACEIs, 68% of the cohort (34 patients) no longer experienced angioedema attacks, while 16 patients (32%) had recurrences. Consistent with the findings of Beltrami et al [28], our data suggest that discontinuation of ACEI may not be sufficient to prevent recurrence of symptoms in a considerable percentage of patients.

We found no significant differences in the incidence of angioedema between the group treated with ARBs (31.8%) and the group treated with other antihypertensive drugs (28.6%). The difference between the 2 groups was not significant, considering that ARBs as replacement therapy were the most prescribed drugs compared with a single antihypertensive, and that of 6 patients with persistent angioedema after the first year of discontinuation of ACEI, only 2 were treated with ARBs (1 with ARB-induced angioedema and no discontinuation of ARBs, 1 with persistent angioedema after discontinuation of ARBs), while 4 patients were treated with other antihypertensive drugs (Figure 1).

Although our sample size was limited, ACEI-AAE is a rare condition, and the data from our population contribute to our understanding of it. We intended to combine real-world data with new laboratory findings to investigate the pathogenesis of ACEI-AAE, even in relation to the presence of hypertension, a condition with pathophysiological processes that also depend on endothelial dysfunction.

The interaction between VEGFs and sPLA₂ in the regulation of vascular permeability has been demonstrated both in vivo and in vitro [29,30]. Recently, our group demonstrated that patients with C1-INH-AAE had elevated plasma levels of VEGFs and Angs, and increased activity of sPLA₂, which we have shown to be attributable to sPLA₂ group IIA, the most representative sPLA₂ in human serum and plasma [4,5].

Similarly, in this study, we aimed to test the same hypothesis on a population of angioedema patients who had not yet been investigated. We observed a higher plasma concentration of VEGF-A and VEGF-C and greater sPLA₂ activity in patients with ACEI-AAE in remission than in healthy controls and ACEI-treated individuals without angioedema (Figure 2A, 2B, 2E). As shown above, the increase in plasma VEGF-A and VEGF-C levels in the ACEI-AAE group did not depend on drug therapy, since the discontinuation of ACEI did not affect the plasma concentration (Figure 4). Therefore, we hypothesized that these patients were characterized by a substrate of increased vascular permeability at baseline due to the increased plasma levels of VEGF-A and VEGF-C. This state, added to ACEI therapy, could increase the probability of developing angioedema compared with patients treated with ACEI but who had lower VEGF levels. The possible origin of the increased plasma levels of VEGF-A and VEGF-C at baseline remains to be clarified.

No differences were found in Ang levels, possibly owing to the action of antihypertensive treatment in reducing circulating levels of Ang in hypertensive patients receiving drug therapy [31,32].

One remarkable finding of the study was the correlation between the different types of ACEI and VEGF-A and

VEGF-C levels and activity of sPLA₂. The highest levels of these proteins were found in patients treated with ramipril, enalapril, and zofenopril. For ramipril and enalapril, this could be associated with the aforementioned greater percentage of prescriptions and long half-life. However, we should emphasize the peculiarities of zofenopril. First, its molecular structure contains a sulfhydryl group, and its activity induces increases in nitrogen monoxide and a decrease in endothelial adhesion molecules with a consequent increase in vasodilation. Second, the previously reported correlation with a higher incidence of ACEI-AAE in male patients [33] was confirmed by our study, where 5 of the 7 ACEI-AAE patients treated with zofenopril were males.

To our knowledge, this is the first study to conduct genetic analyses of ACEI-AAE patients with the aim of investigating cases of misdiagnosed hereditary angioedema. In the last few years, multiple studies have shown newly discovered forms of hereditary angioedema with incomplete penetrance and variable clinical presentations that may hamper diagnosis [8-12]. In our study, genetic screening did not reveal any diagnostic errors. Indeed, all patients classified as ACEI-AAE were actually affected by the acquired form of angioedema. Cases initially classified as ACEI-AAE but later revealed to be inherited are very rare. The high cost of the genetic analysis procedure leads us not to recommend genetic screening for patients with a history suggestive of ACEI-AAE.

The study is limited by its small ACEI-AAE patient population (51 patients) and the low number of patients for whom laboratory data were available (43 patients). However, gathering a large sample population for this type of investigation is difficult because ACEI-AAE is a rare condition.

In conclusion, our results demonstrate high baseline levels of VEGF-A, VEGF-C, and sPLA₂ in patients with ACEI-AAE, suggesting a predisposition to vascular permeability that could play a role in the development of an angioedema attack, as ACEIs lead to an increase in bradykinin levels due to reduced catabolism. However, we observed no correlations between plasma levels of VEGF-A and VEGF-C, sPLA₂ activity, and clinical features.

Acknowledgments

This publication arose in part from a mentor-mentee collaboration resulting from the 2017 round of the European Academy of Allergy and Clinical Immunology (EAACI) Junior Member Mentorship Programme.

The authors wish to thank Rossella Iannone and SEEd Medical Publishers (Turin, Italy) for writing and editorial support during the preparation of this manuscript.

Funding

CSL Behring Italy funded the medical writing support but made no contribution to the contents.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentation

These data were presented at the European Academy of Allergy & Clinical Immunology (EAACI) Congress 2019, Lisbon (June 1-5, 2019).

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■ *Manuscript received November 4, 2019; accepted for publication January 24, 2020.*

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