Angiotensin-converting enzyme inhibitor-associated angioedema in a cohort of Caucasian patients: from bed to bench

Running title: ACEI-AAE in a Caucasian cohort

Carucci L[1], Bova M[2], Petraroli A[2], Ferrara AL[2], Sutic A[3], de Crescenzo G[4], Cordisco G[5], Margaglione M[5], Gambardella J[6], Spadaro G[1,2], Genovese A[1-2], Loffredo S[2,7]

[1]Post-Graduate Program in Clinical Immunology and Allergy, University of Naples Federico II, Naples, Italy.
[2]Department of Translational Medical Sciences and Interdepartmental Center for Research in Basic and Clinical Immunology Sciences, University of Naples Federico II, Naples, Italy.
[3]Division of Clinical Immunology, Allergology and Rheumatology, Department of Internal Medicine, University of Zagreb School of Medicine, University Hospital Dubrava, Zagreb, Croatia.
[4]Division of Clinical Immunology and Allergy, Sant'Anna and San Sebastiano Hospital, Caserta, Italy.
[5]Medical Genetics, Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy.
[6]Department of Medicine and Surgery, University of Salerno, Salerno, Italy.
[7]Institute of Experimental Endocrinology and Oncology G. Salvatore, National Research Council, Naples, Italy

Corresponding Author
Maria Bova
Department of Translational Medical Sciences and Interdepartmental Center for Research in Basic and Clinical Immunology Sciences
University of Naples Federico II
Via S. Pansini 5
80131 Naples, Italy
E-mail: bovamaria@virgilio.it

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0458

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

Abstract

Background: Angiotensin-converting enzyme inhibitor-associated angioedema (ACEI-AAE) occurs in 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), angiopoietins 1 and 2 (Ang1/Ang2), and secretory phospholipases A_2 ($sPLA_2$) in ACEI-AAE pathogenesis.

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

Results: 51 patients (57% male) were diagnosed with ACEI-AAE. The average time to symptom onset from the start of ACEI therapy was 3 years (range 30 days-20 years). The most commonly affected sites were 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 $sPLA_2$ plasma levels were higher in ACEI-AAE patients than in the controls. Ang1/2 concentrations were not modified. No mutations were detected in the genes analyzed.

Conclusions: Our data suggest that sartans can be a safe therapeutic alternative in ACEI-AAE patients. Increased concentrations of VEGF-A, VEGF-C, and $sPLA_2$ in ACEI-AAE patients suggest a possible role of these mediators in ACEI-AAE pathogenesis.

Key words: Angiotensin-Converting Enzyme Inhibitor. Angioedema. Vascular Endothelial Growth Factor C. Vascular Endothelial Growth Factor A. Phospholipases A2. Genetic analysis. C1-inhibitor. Biomarkers.

doi: 10.18176/jiaci.0458

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

Background

Angioedema (AE) is localized and self-limiting edema involving the subcutaneous tissues and submucosa, and it can be an expression of either allergic or non-allergic diseases. Angiotensin-converting enzyme inhibitor-associated AE (ACEI-AAE), a form of acquired AE without wheals [1,2], is a rare adverse effect related to ACEI treatment that occurs in 0.1%-0.7% of ACEI-treated 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 (e.g., bradykinin, substance P), which may result in their accumulation and a consequent increase of vascular permeability. However, uncertainties regarding ACEI-AAE pathogenesis remain, as only a small percentage of ACEI-treated patients experience AE, and the time to symptoms onset 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 phospholipases A_2 ($sPLA_2$) [5] in patients with hereditary AE 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 AE, such as ACEI-AAE, may elucidate the pathogenesis and stratification of patients treated with ACEIs who are potentially at risk of developing this adverse event. Recently, Firinu et al. demonstrated the importance of conducting genetic investigations in patients with different forms of idiopathic AE for accurate diagnostic work-ups [6]. When clinicians encounter a recurrent form of AE with normal C1-INH levels and no specific diagnostic biomarkers, distinguishing between hereditary and acquired AE is difficult. Family history and age of onset may occasionally be informative because there are forms of AE where family history is negative, age of onset is advanced, and ACEI treatment triggers the 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 can describe up to

90% of asymptomatic AE carriers [6,8].The absence of family history has been observed in other recently discovered hereditary forms associated with mutations in *PLG* gene [9-11] or *ANGPT1* gene [12], in which patients were initially diagnosed with acquired idiopathic AE that was later revealed as hereditary.

This study assessed the clinical and laboratory features of a Caucasian cohort of ACEI-AAE patients. The investigations included the possible risk and/or protective factors for ACEI-AAE development, possible correlations between the clinical features and laboratory parameters (levels of VEGFs and Angs, and $sPLA_2$ activity) of ACEI-AAE patients, risk of angiotensin II receptor blockers(ARBs)-induced AE in patients with a history of ACEI-AAE, and the utility of extensive genetic screening to identify rare hereditary AE forms.

METHODS

Participants

The study population was a cohort of Caucasian patients with ACEI-AAE from two AE reference centers (Center for the diagnosis and therapy of the angioedema, University Federico II, Naples, Italy; Division of Clinical Immunology, Allergy and Rheumatology, University Hospital Dubrava, Zagreb, Croatia). Healthy volunteers and ACEI-treated subjects without AE were enrolled as controls for the evaluation of vascular permeability factors (VEGFs, $sPLA_2$, and Angs). Genetic analyses to detect mutations in *SERPING1*, *ANGPT1*, *PLG*, and *F12* gene were performed in a subset of patients.

Diagnoses were based on clinical symptoms, plasma levels and 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 compliance with the Helsinki Declaration.

Blood sampling

Blood was collected during routine diagnostic procedures, and all patients gave oral informed consent that the remaining plasma could be used for research purposes. The remaining plasma samples of consenting patients were labeled with codes that were recorded into a data sheet. The control patients had been

doi: 10.18176/jiaci.0458

referred for a routine medical checkup and volunteered for the study by giving 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 AE attack (remission sample). The samples were collected via clean venipunctures and minimal stasis using 3.2% sodium citrate. After centrifugation at 2000 g for 20 min at room temperature, the plasma was divided into aliquots and stored at -80°C until tested.

Complement system

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

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 System, Minneapolis, USA) according to the manufacturer's instructions. The ELISA sensitivity was 31.1-2,000 pg/ml for VEGF-A, 62-4,000 pg/ml for VEGF-C, 156.25-10,000 pg/ml for Ang1, and 31.1-4,000 pg/ml for Ang2.

Functional test of $sPLA_2$

The activity of $sPLA_2$ in the plasma of ACEI-AAE patients, healthy controls and subjects treated with ACEIs without angioedema was measured by EnzChek® phospholipase A_2 assay (Life Technologies, Carlsbad, California, USA). Briefly, an $sPLA_2$ substrate cocktail consisting of 7-hydroxycoumarinyl-arachidonate (0.3 mM), 7-hydroxycoumarinyl-linolenate (0.3 mM), hydroxycoumarinyl-6 heptenoate (0.3 mM), dioleoylphosphatidylcholine (DOPC) (10 mM), and dioleoylphosphatidylglycerol (DOPG) (10 mM) was prepared in ethanol. Liposomes were formed by gradually adding 77 µl of substrate/lipid cocktail to 10 ml of PLA_2 buffer (50 mMTris–HCl, 100 mMNaCl, and 1 mM $CaCl_2$) while stirring rapidly over 1 min with a magnetic stirrer. Fluorescence (excitation at 360 nm and emission at 460 nm) was measured, and the specific activity (relative fluorescent units/ml) was calculated for each sample. Fifty-microliter aliquots of plasma from ACEI-AAE patients, subjects treated with ACEIs without angioedema, and healthy controls

doi: 10.18176/jiaci.0458

were added to 96-well plates, and $sPLA_2$ activity in the samples was measured by adding 50 µl of substrate cocktail.

Genetic screening

Direct DNA sequencing was conducted with the Big Dye® Terminator Cycle Sequencing Kit 3.1 (Applied Biosystems, Forster City, CA USA). The obtained sequences were compared with consensus sequences from specific databases (Ensembl 2011).

Statistical analysis

Collected data were processed using statistical software (GraphPadPrism 5, GraphPad Software, San Diego, CA, USA), and were tested for normality using the D'Agostino-Pearson normality test. Data with a normal distribution determined at a significance level of 0.05 were subjected to parametric tests. Non-parametric tests were used for non-normally distributed data. Two-tailed t-tests were performed for independent samples, and two-tailed Mann-Whitney tests were performed as indicated in the figure legends. The correlation between two variables was assessed by the Pearson or Spearman test and reported as a coefficient of correlation (*r*). A *P* value ≤ 0.05 was considered statistically significant.

RESULTS

Clinical characteristics of the participants

A total of 51 Caucasian patients with ACEI-AAE were included in this study. As controls, 86 healthy subjects and 20 ACEI-treated subjects without AE were enrolled. Table I summarizes the clinical features of all participants.

The median interval between the start of ACEI therapy and the onset of AE was 3 years (range 3 days-20 years). The median duration of ACEI therapy was 4 years (range 3 days-20 years).The majority of patients (39.2%) experienced the first attack of acute AE between the first and fifth year of beginning ACEI therapy, 35.3% after five 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 AE, ACEI prescriptions included ramipril (70%), lisinopril (15%), enalapril (10%), and zofenopril (5%).

Measuring from the initiation of the ACEI therapy, the median number of total AE attacks per patient was 3 (range 1-180). An average AE attack frequency per year could not be estimated as 31.4% of patients discontinued ACEI after the first or second episode.

The distribution of 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 non-cumulative percentages, meaning patients may have exhibited attacks involving multiple locations simultaneously.

The average AE attack duration 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, the patients received follow-up assessments for up to 12 years, with a minimum follow-up time of 6 months. Ninety-eight percent of patients suspended ACEI use, whereas 2% (1 patient) did not discontinue the drug and their AE attacks persisted. The control group with ACEI-treated subjects without AE was treated with ACEI for a minimum time of 6 months to 20 years.

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

For antihypertensive replacement therapy, we observed that 44% (22/50) switched to ARBs, while 56% of patients (28/50) started other antihypertensive drugs or discontinued antihypertensive therapy (36% to calcium antagonists, 14% to no therapy, and 8% to beta-blockers, alpha-blockers, or diuretics). We found that 9/22 patients developed AE during ARBs treatment but for 2 patients AE was not related to ARBs (1 with spontaneous resolution despite the continuation of ARBs; 1 with persistent AE after ARBs

discontinuation). Ultimately, ARBs-induced AE were confirmed in 31.8% of patients (7/22). The prevalence of AE in patients treated with other antihypertensive drugs was 28.6% (8/28).

Among the patients with persistent attacks, the attacks recurred in 50% of patients treated with either 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, and displayed no improvement of symptoms. Three patients received intramuscular adrenaline as adjunctive therapy but did not report positive effects on the duration of the episodes, or side effects related to the drug. One patient received icatibant as add-on therapy but did not show a significant reduction in attack duration.

Laboratory parameters of the population

Figures 2A and 2B show that VEGF-A and VEGF-C plasma levels in patients with ACEI-AAE were higher than in the healthy controls or ACEI-treated subjects without AE. Median VEGF-A values were 21.7 (0-70.9) *vs* 0 (0-0) *vs* 0 (0-0) pg/ml (interquartile ranges), 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 subjects without AE (Figure 2C-2D).

The $sPLA_2$ enzymatic activity was approximately 3 times higher in patients with ACEI-AAE than in healthy controls or ACEI-treated subjects without AE. 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 AE 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 $sPLA_2$ enzymatic activity within each group. Patients treated with enalapril, ramipril, or zofenopril had higher levels of VEGF-A, VEGF-C, and $sPLA_2$ than the controls (Figure 3).

We also investigated possible correlations between laboratory parameters and clinical features of AE attacks. We found no significant relationship between plasma concentrations of VEGF-A and VEGF-C, activity of $sPLA_2$, and time between the onset of therapy and symptoms onset. Furthermore, no correlation was observed between the laboratory parameters and age of symptoms onset, duration of episodes, or number of sites involved.

To determine whether ACEI therapy influenced the patients' plasma levels of VEGF-A and VEGF-C and the activity of $sPLA_2$, we assessed the relationship between the laboratory parameters and the duration from plasma draw to ACEI therapy discontinuation. However, the results did not reveal a significant correlation between this clinical feature and variations in the laboratory parameters (Figure 4).

In further analysis, the patients were divided into three groups according to their persistence of AE attacks after ACEI discontinuation. The groups included patients with persistence of the attacks after ACEI discontinuation, patients who experienced attacks within 1 year after ACEI discontinuation, and patients with a resolution of symptoms following therapy suspension (no attacks). We then analyzed the laboratory parameters of the three patient groups. Figure 5 shows that the plasma concentrations of VEGF-A and VEGF-C, and the activity of $sPLA_2$ did not vary in the three groups analyzed.

Genetic characteristics of the population

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

DISCUSSION

AE is a rare but potentially life-threatening side effect of ACEI treatment. The diagnostic evaluation and treatment of acute AE are challenging. Due to the vast number of patients who are treated with ACEIs, identifying those potentially at risk of developing ACEI-AEE is a highly relevant and complicated topic.

doi: 10.18176/jiaci.0458

Here, we report the clinical characteristics of a Caucasian patient cohort and highlight some correlations that only partially confirm previous data regarding the possible roles of the female sex, smoking, and age over 65 years as risk factors associated with ACEI-AAE. Our results differ from the literature in terms of correlations with sex, as most of our patients were male [13-15]. Furthermore, there was a nearly uniform distribution between smokers and non-smokers in our study (45% vs 55%). A correlation between ACEI-AAE and age was present but not strong, although approximately 50% of our patients were ≥ 65 years of age. Diabetes is commonly reported in the literature as a protective factor, which is congruent with our results (14% patients with diabetes vs. 86% without), supporting the role of DPP-4 as an important enzyme in bradykinin (BK) catabolism [16-18]. A more controversial topic is the correlation between IgE-mediated diseases and ACEI-AAE. While some studies consider the history of atopy and drug allergy as risk factors for ACEI-AAE [13,14], others do not find a significant relationship between the two mechanisms [15]. In our study's population, the 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 developing histamine-mediated symptoms, such as pruritus and rash, during episodes of AE. Of the 8 patients suffering from drug allergy in our cohort, 50% exhibited associated symptoms during AE episodes, as if the tendency of these patients to develop histamine-mediated drug reactions conferred susceptibility to the release of histamine during episodes of BK-mediated AE. Some authors propose that an initial histamine release might trigger BK-mediated AE attacks, although this hypothesis is yet to be supported by sufficient evidence [19,20]. However, there is evidence to support the notion that classic antiallergic drugs such as antihistamines and corticosteroids are not effective in ACEI-AAE treatment, and the histamine contribution is present but not significant.

The time frame for ACEI-AAE presentation 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 agrees with the trend demonstrated by various studies [21,22].

The ACEI most correlated with the onset of AE in our cohort was ramipril (27.5%), followed by enalapril (23.5%) and lisinopril (17.6%). Although in Italy these drugs, especially enalapril, are among the most prescribed in their class [23], a potentially higher risk of developing ACEI-AAE in patients taking such drugs compared with that in patients taking other ACEIs cannot be excluded. This increased risk may be partially explained by the prolonged half-life of enalapril (11 hours), ramipril (15 hours), and lisinopril (12 hours), along with the greater potency expressed as IC50 (concentration required to inhibit 50% of the enzymatic activity) of these ACEIs compared to that of others [24]. Montoro et al. showed that the use of enalapril was associated with an increased risk of developing ACEI-AAE compared with the use of other ACEIs [25]. Other studies showed a greater percentage of ACEI-AAE occurrence in patients treated with lisinopril [22,26,27]. As a possible explanation, the authors report that lisinopril was the most prescribed drug in their patient population, although lisinopril is also part of the triad of ACEIs with longer half-life and greater potency.

After discontinuation from ACEIs, 68% of our cohort (34 patients) no longer experienced AE attacks, while 16 patients (32%) had AE recurrences. These data, in accordance with a study by Cicardi et al. [28], suggest that discontinuation from ACEI may not be sufficient to prevent symptom recurrence in a considerable percentage of patients.

In our study, there were no significant differences in AE incidence observed between the group treated with ARBs (31.8%) and the groups treated with other antihypertensive drugs (28.6%). The difference between two groups were not significant considering that ARBs as replacement therapy were the most prescribed drugs compared to single antihypertensive, and that among 6 patients with persistent AE after the first year of ACEI discontinuation, only 2 patients were treated with ARBs (1 with ARBs-induced AE and no ARBs discontinuation, 1 with persistent AE after ARBs discontinuation) while 4 patients were treated with other antihypertensive drugs (Figure 1).

Although our sample size was limited, the disease of interest is a rare pathology, and the data from our population contribute to the understanding of ACEI-AAE. Our study 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 physiopathological processes that also depend on endothelial dysfunction**.**

The interaction between VEGFs and $sPLA_2$ 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, Angs, and $sPLA_2$, particularly those of the IIA group (hGIIA), in the periods without AE attacks [4,5].

Similarly, in this study, we aimed to test the same hypothesis on a population of patients suffering from AE who were not yet investigated. We observed a higher plasma concentration of VEGF-A and VEGF-C and greater $sPLA_2$ enzymatic activity in patients with ACEI-AAE in remission compared to that in healthy controls and ACEI-treated patients without AE (Figure 2A, 2B, 2E). As shown above, the increase in VEGF-A and VEGF-C plasma levels in 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 in these patients 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 contribute to increasing the probability of developing AE compared to patients treated with ACEI but with lower VEGFs levels. The possible origin of the increased plasma levels of VEGF-A and VEGF-C at baseline remains to be clarified.

No differences in the levels of Angs were found. This could be explained by 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 VEGF-A, VEGF-C, and $sPLA_2$ levels and different types of ACEI. The highest levels of these proteins were found in patients treated with ramipril, enalapril, or zofenopril. For ramipril and enalapril, this could be associated with the aforementioned greater percentage of prescriptions and prolonged half-life. However, there are some peculiarities regarding zofenopril that should be emphasized. First, its molecular structure contains a sulfhydryl group, and its activity induces increases in nitrogen monoxide (NO) and the reduction of endothelial adhesion

doi: 10.18176/jiaci.0458

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 that conducted genetic analyses of ACEI-AAE patients to investigate cases of misdiagnosed hereditary AE. In the last few years, multiple studies have shown newly discovered forms of hereditary AE with incomplete penetrance and variable clinical presentations that may interfere with accurate diagnoses [8-12]. In our study, genetic screening did not reveal any diagnostic errors. All patients classified as ACEI-AAE in our cohort were actually affected by the acquired form of AE. Cases initially classified as ACEI-AAE but later revealed as the inherited form are very rare, and considering the economic burden of the genetic analysis procedure, we assert that genetic screening is not necessary 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 subjected to laboratory evaluations (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 basal levels of VEGF-A, VEGF-C, and $sPLA_2$ in ACEI-AAE patients, suggesting a basal predisposition to vasopermeability in ACEI-AAE patients that could play a role in the development of an AE attack, as ACEIs lead to an increase in bradykinin levels due to reduced catabolism. However, no correlations between plasma levels of VEGF-A and VEGF-C, $sPLA_2$ activity, and clinical features were observed.

Acknowledgments

This publication partially arose 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 medical writing support but made no contribution to the contents.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This publication partially arose 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 medical writing support but made no contribution to the contents.

Conflict of interest

The authors declare that they have no conflicts of interest.

REFERENCES

1. 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.
2. Cicardi M, Suffritti C, Perego F, Caccia S. Novelties in the Diagnosis and Treatment of Angioedema. J Investig Allergol Clin Immunol. 2016;26:212-21.
3. Bas M. The Angiotensin-Converting-Enzyme-Induced Angioedema. Immunol Allergy Clin North Am. 2017;37:183-200.
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. Firinu D, Loffredo S, Bova M, Cicardi M, Margaglione M, Del Giacco S. The role of genetics in the current diagnostic workup of idiopathic non-histaminergic angioedema. Allergy. 2019;74:810-12.
7. Grant NN, Deeb ZE, Chia SH. Clinical experience with angiotensin-converting enzyme inhibitor-induced angioedema. Otolaryngol Head Neck Surg. 2007;137:931-5.
8. Veronez CL, Serpa FS, Pesquero JB. A rare mutation in the F12 gene in a patient with ACE inhibitor-induced angioedema. Ann Allergy Asthma Immunol. 2017;118:743-5.
9. Bork K, Wulff K, Steinmüller-Magin L, Braenne I, Staubach-Renz P, Witzke G, et al. Hereditary angioedema with a mutation in the plasminogen gene. Allergy. 2018;73:442-50.
10. Germenis AE, Loules G, Zamanakou M, Psarros F, González-Quevedo T, Speletas M, et al. On the pathogenicity of the plasminogen K330E mutation for hereditary angioedema. Allergy. 2018;73:1751-3.

11. Yakushiji H, Hashimura C, Fukuoka K, Kaji A, Miyahara H, Kaname S, et al. A missense mutation of the plasminogen gene in hereditary angioedema with normal C1 inhibitor in Japan. Allergy. 2018;73:2244-7.
12. 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 ClinImmunol. 2018;141:1009-17.
13. Kostis JB, Kim HJ, Rusnak J, Casale T, Kaplan A, Corren J, et al. Incidence and characteristics of angioedema associated with enalapril. Arch Intern Med. 2005;165:1637-42.
14. Woodard-Grice AV, Lucisano AC, Byrd JB, Stone ER, Simmons WH, Brown NJ. Sex dependent and race-dependent association of XPNPEP2 C-2399A polymorphism with angiotensin-converting enzyme inhibitor-associated angioedema. Pharmacogenet Genomics. 2010;20:532-6.
15. Rasmussen ER, von Buchwald C, Wadelius M, Prasad SC, Kamaleswaran S, Ajgeiy KK, et al. Assessment of 105 Patients with Angiotensin Converting Enzyme-Inhibitor Induced Angioedema. Int J Otolaryngol. 2017;2017:1476402.
16. Scott SI, Andersen MF, Aagaard L, Buchwald CV, Rasmussen ER. Dipeptidyl Peptidase-4 Inhibitor Induced Angioedema - An Overlooked Adverse Drug Reaction? Curr Diabetes Rev. 2018;14:327-33.
17. Ahmad S, Wang L, Ward PE. Dipeptidyl(amino)peptidase IV and aminopeptidase M metabolize circulating substance P in vivo. J Pharmacol Exp Ther. 1992;260:1257-61.
18. Byrd JB, Shreevatsa A, Putlur P, Foretia D, McAlexander L, Sinha T, et al. Dipeptidyl peptidase IV deficiency increases susceptibility to angiotensin-converting enzyme inhibitor-induced peritracheal edema. J Allergy Clin Immunol. 2007;120:403-8.
19. Madsen F, Attermann J, Linneberg A. Epidemiology of non-hereditary angioedema. Acta Derm Venereol. 2012;92:475-9.

20. Zuraw BL, Bork K, Binkley KE, Banerji A, Christiansen SC, Castaldo A, et al. Hereditary angioedema with normal C1 inhibitor function: consensus of an international expert panel. Allergy Asthma Proc. 2012;33Suppl 1:S145-56.
21. Brown T, Gonzalez J, Monteleone C. Angiotensin-converting enzyme inhibitor-induced angioedema: A review of the literature. J ClinHypertens (Greenwich). 2017;19:1377-82.
22. Chan NJ, Soliman AM. Angiotensin converting enzyme inhibitor-related angioedema: onset, presentation, and management. Ann Otol Rhinol Laryngol. 2015;124:89-96.
23. Osservatorio Nazionale sull'impiego dei Medicinali. L'uso dei farmaci in Italia. Rapporto Nazionale 2017. Roma: Agenzia Italiana del Farmaco, 2018.
24. Edeki T, Johnston A, Li KamWa E, Turner P. Enalapril pharmacokinetics and ACE inhibition, following single and chronic oral dosing. Int J Clin Pharmacol Ther. 1994;32:142-6.
25. Montoro de Francisco A, García-Luque A, Fernández M, Puerro M. Side effects of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists: are we facing a new syndrome. Am J Cardiol. 2012;110:1552-3.
26. Banerji A, Clark S, Blanda M, LoVecchio F, Snyder B, Camargo CA Jr. Multicenter study of patients with angiotensin-converting enzyme inhibitor-induced angioedema who present to the emergency department. Ann Allergy Asthma Immunol. 2008;100:327-32.
27. Al-Khudari S, Loochtan MJ, Peterson E, Yaremchuk KL. Management of angiotensin-converting enzyme inhibitor-induced angioedema. Laryngoscope. 2011;121:2327-34.
28. Beltrami L, Zanichelli A, Zingale L, Vacchini R, Carugo S, Cicardi M. Long-term follow-up of 111 patients with angiotensin-converting enzyme inhibitor-related angioedema. J Hypertens. 2011;29:2273-7.
29. Sirois MG, Edelman ER. VEGF effect on vascular permeability is mediated by synthesis of platelet-activating factor. Am J Physiol. 1997;272:H2746-56.

30. Bernatchez PN, Winstead MV, Dennis EA, Sirois MG. VEGF stimulation of endothelial cell PAF synthesis is mediated by group V 14 kDa secretory phospholipase A2. Br J Pharmacol. 2001;134:197-205.
31. Nadar SK, Blann A, Beevers DG, Lip GY. Abnormal angiopoietins 1&2, angiopoietin receptor Tie-2 and vascular endothelial growth factor levels in hypertension: relationship to target organ damage [a sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT)]. J Intern Med. 2005;258:336-43.
32. Nadar SK, Blann AD, Lip GY. Plasma and platelet-derived vascular endothelial growth factor and angiopoietin-1 in hypertension: effects of antihypertensive therapy. J Intern Med. 2004;256:331-7.
33. Franconi F, Omboni S, Ambrosioni E, Reggiardo G, Campesi I, Borghi C. Effects of treatment with zofenopril in men and women with acute myocardial infarction: gender analysis of the SMILE Program. PLoS One. 2014;9:e111558.

Table 1. Clinical characteristics of participants.

	ACEI-AAE patients (n = 51)	Healthy (n = 86)	ACEI-treated without AE (n = 20)
Male : Female, n. (%)	29 : 22 (56.8 : 43.2)	46 : 40 (53.5 : 46.5)	11 : 9 (55 : 45)
Age at diagnosis, median (range) - years	64 (42-90)	NA	NA
Age onset symptoms, median (range) - years	63 (42-80)	NA	NA
Hospitalization, n. (%)	32 (62.7)	NA	NA
Associated symptoms, n. (%) • Itching, n (%) • Rash, n. (%)	12 (23.5) • 9 (75) • 3 (25)	NA	NA
Comorbidities • Diabetes, n. (%) • Atopia, n. (%)	 • 7 (14) • 12 (23.5)	NR	 • 9 (56.2)* • 3 (21.4)**
Allergies to other drugs, n. (%)	8 (15.7)	NR	3 (21.4)**
Smoking, n. (%)	23 (45)	NR	6 (30)
Family history for ACEI-AAE, n. (%)	3 (5.9)	NA	1 (5)

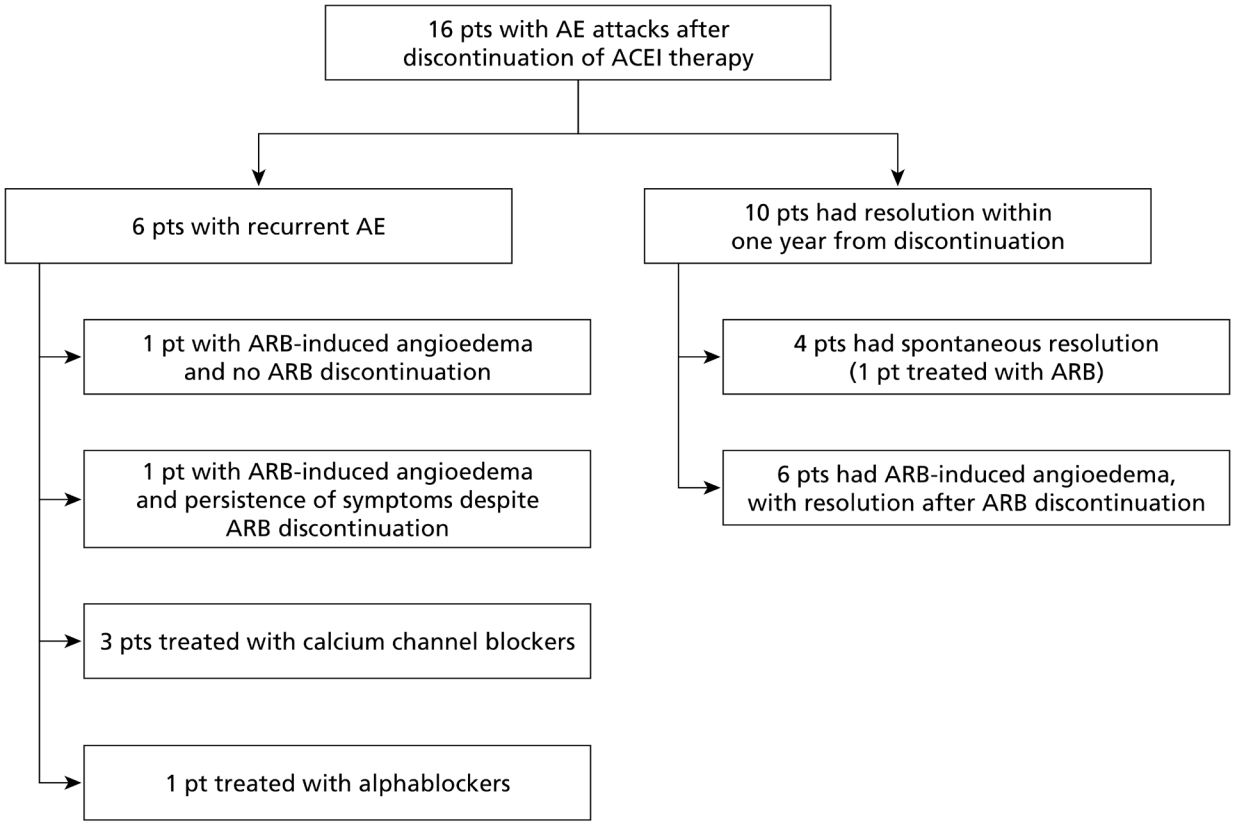
NA = not applicable; NR = not reported

* Reported data for 16 patients

** Reported data for 14 patients

FIGURE

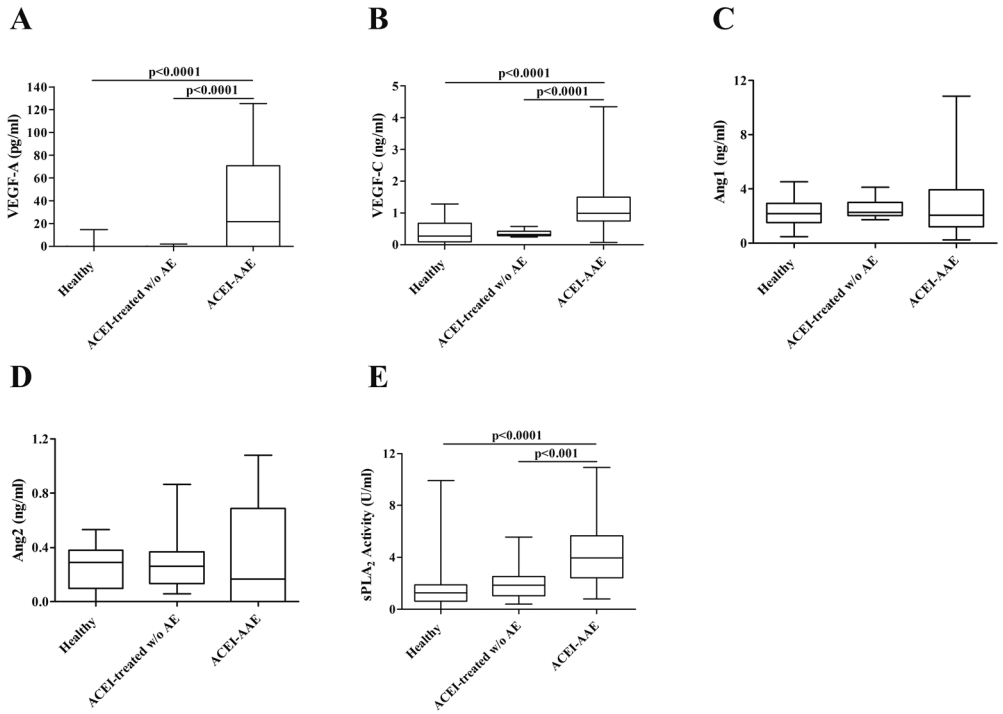
Figure 1. Patients (pts) with persistent angioedema (AE) attacks after discontinuation of ACEI therapy



ACEI = Angiotensin-converting-enzyme inhibitor

ARB = Angiotensin II receptor blockers

Figure 2. Plasma concentrations of VEGF-A, VEGF-C, Ang1, Ang2 and $sPLA_2$ in study population.



Plasma VEGF-A (A), VEGF-C (B), Ang1 (C), Ang2 (D) and $sPLA_2$ (E) in controls (Healthy), ACEI-treated subjects without angioedema (ACEI-treated w/o AE) and patients with ACEI-AAE in remission. Data are shown as the median (horizontal black line), the 25thand 75th percentiles (boxes) and the 5th and 95th percentiles (whiskers) of controls and patients.

VEGF-A = vascular endothelial growth factor A

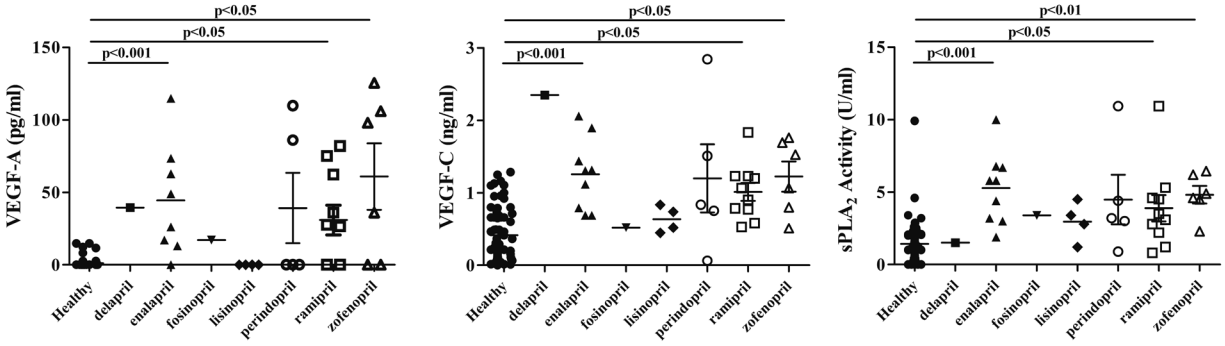
VEGF-C = vascular endothelial growth factor C

Ang1 = angiopoietins 1

Ang2 = angiopoietins 2

$sPLA_2$ = secretory phospholipases A_2

Figure 3. Plasma levels of VEGF-A and VEGF-C, and plasma activity of $sPLA_2$ in healthy subjects and ACEI-AAE patients by type of ACEI

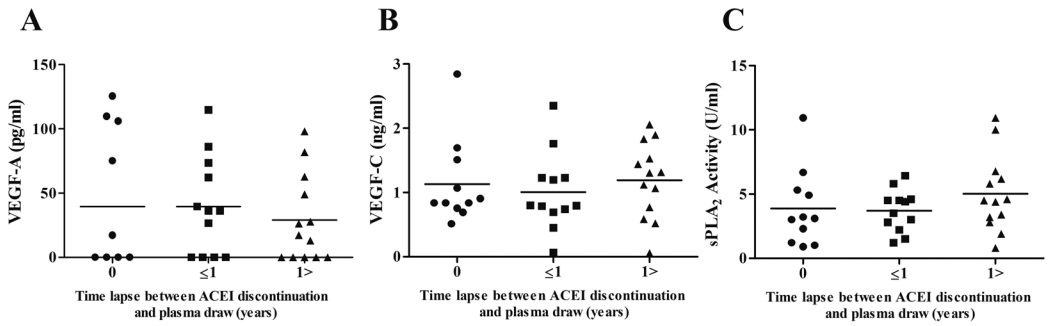


VEGF-A = vascular endothelial growth factor A

VEGF-C = vascular endothelial growth factor C

$sPLA_2$ = secretory phospholipases A_2

Figure 4. Correlation between plasma levels of VEGF-A, VEGF-C, and $sPLA_2$ and the time between ACEI discontinuation and plasma draw (years)



0 = plasma draw at ACEI discontinuation

≤1 = plasma draw within1 year from ACEI discontinuation

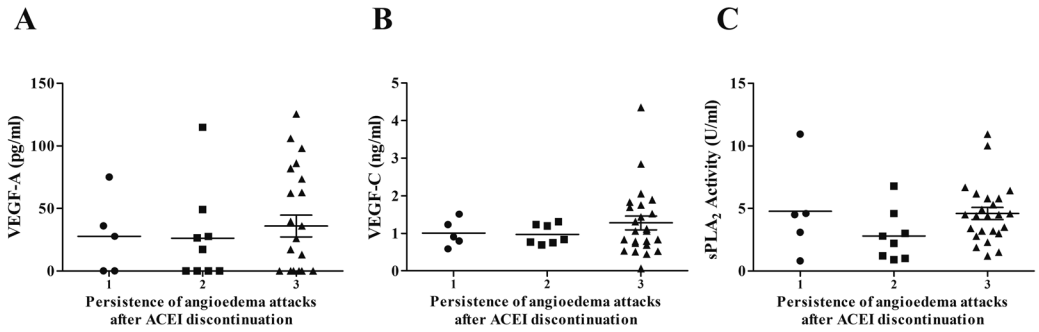
>1 = plasma draw more than 1 year from ACEI discontinuation

VEGF-A = vascular endothelial growth factor A

VEGF-C = vascular endothelial growth factor C

$sPLA_2$ = secretory phospholipases A_2

Figure 5.Correlation between the plasma levels of VEGF-A, VEGF-C and $sPLA_2$ and the persistence of angioedema attacks after ACEI discontinuation



1 = persistence of the attacks

2 = attacks within 1 year after ACEI discontinuation

3 = no attacks after ACEI discontinuation

VEGF-A = vascular endothelial growth factor A

VEGF-C = vascular endothelial growth factor C

$sPLA_2$ = secretory phospholipases A_2