

BIOBRAD Study: The Search for Biomarkers of Bradykinin-Mediated Angio-Oedema Attacks

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Key Words

Angio-oedema · Bradykinin · Histamine · D-dimers · Biomarkers · VE-cadherin · Complement

Abstract

Background: The aetiology of angio-oedema (AE) is difficult to determine; however, it is essential in emergency situations when two major contexts may be present: mast cell-mediated AE and bradykinin-mediated AE. Different forms of AE are currently distinguished based on clinical criteria (spontaneous duration of the attack, presence of concomitant or late-appearing superficial urticaria, history of atopy, and others), but specific biomarkers could improve patient management. **Objective:** In this prospective study, potential biomarkers have been identified, and their statistical characteristics were examined. **Methods:** Samples were taken on day 0 (D0) and D7 for 3 patient groups (n = 11 each): bradykinin-mediated AE [peripheral site of attack, ear, nose, throat (ENT), and abdominal involvement], mast

cell-mediated AE, and non-bradykinin-mediated abdominal pain. **Results:** Assay of the potential biomarkers revealed no significant differences in C1 inhibitor and C4 levels. In contrast, D-dimer levels peaked during bradykinin-mediated AE attacks (median 2.2 mg/l at D0 vs. 0.52 mg/l at D7; $p < 10^{-3}$) as well as during mast cell-mediated AE attacks (1.97 vs. 0.65 mg/l; $p = 0.04$) and were high in bradykinin-mediated AE compared to the control group (0.69 mg/l; $p = 0.01$). A threshold value of 0.62 mg/l was found to have a negative predictive value of 100% for bradykinin-mediated AE compared to other causes of abdominal pain (group 3). Circulating VE-cadherin levels were also increased during an attack (1,990 at D0 vs. 1,566 ng/ml at D7; $p = 0.01$), but could not distinguish between bradykinin-mediated and mast cell-mediated AE, like D-dimers. **Conclusions:** Exploration of changes in fibrinolysis-related markers (particularly D-dimers) is thus promising for the diagnosis of AE attacks in difficult-to-diagnose abdominal forms, although it was not able to differentiate between bradykinin and mast cell-mediated AE.

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Introduction

Angio-oedema (AE) is a clinical syndrome characterized by subcutaneous or submucosal swelling, which is localized, transitory, and regresses without sequelae [1]. AE is induced through the sudden localized release of vasodilators, which cause vascular hyperpermeability. The classification of AE relies in a large part on the nature of the mediators causing it [2]. The majority of AE cases are linked to the release of histamine, mainly by mastocytes, whereas bradykinin-mediated AE cases are rarer. These are linked to the activation of the kallikrein-kinin pathway and to the release of bradykinin, by excessive activation of the kinin pathway and/or by reduced degradation of pathway intermediates. The proteins involved in the contact phase of coagulation are responsible for activating the kinin pathway; factor XII (or Hageman factor) is a key player in this phenomenon [3]. The protein is activated by an indicator of stress at the surface of vascular endothelial cells and triggers activation of plasmin and the kallikrein-kinin pathway (to produce bradykinin) [4].

Bradykinin-mediated AE is a group of diseases [2, 3] including hereditary AE (HAE) and acquired AE. HAE can subsequently be divided into 3 types, caused by C1 inhibitor (C1INH) deficiency (type I and II) and HAE with factor XII mutations, or of unknown origin (type III). Acquired AE is associated with the use of angiotensin-converting enzyme inhibitors (ACEi), responsible for the inhibition of bradykinin degradation by ACE. HAE type I and II and ACEi-induced AE are associated with a high level of morbidity-mortality and have an effect on quality of life similar to that experienced by patients with asthma [5]. When oedema affects the upper respiratory tract, death occurs in 25% of cases that go untreated [6]. This oedema does not respond to treatments given for mast cell-induced AE (corticosteroids, antihistamines, adrenalin). Effective, specific treatments for bradykinin-mediated AE do exist, but they are expensive and should therefore be reserved for treatment of the most severe attacks (ENT and digestive tract involvement) [7]. Finally, idiopathic AE is diagnosed when all other causes have been excluded; this form of AE is not represented in our study.

Most often, clinicians are seeking to distinguish mast cell-mediated AE from bradykinin-mediated AE while generally using some clinical elements to make the distinction between these two aetiologies. The most discriminating element is the presence of superficial urticaria (not necessarily present at the time of the attack) which is indicative of mast cell-induced AE; however, this symp-

tom is not always present [8]. Especially in the abdominal form, it is difficult to distinguish between AE and abdominal pain of other aetiology, and no biomarkers are available in clinical practice.

When a patient is admitted to the emergency services with AE involving the upper respiratory tract or the abdominal region, it is essential to be able to rapidly diagnose bradykinin-mediated AE so as to administer an appropriate treatment as soon as possible. Currently, no specific marker of AE attacks has been identified to help make the therapeutic decision, but many studies have attempted to identify one [9]. These studies have highlighted several candidates [9]: markers related to endothelial stress (VE-cadherin, E-selectin, endothelin-1, as well as activity and levels of von Willebrand factor), which are significantly increased during an AE attack; the complement system; the contact phase of coagulation and fibrinolysis, and inflammatory proteins (C-reactive protein, interleukins, fibrinogen, and others). The diagnostic utility of these markers must be validated by prospective studies. Therefore, in this article, we present a prospective study assessing the diagnostic advantages of the various biomarkers available in current practice and the utility of their assay during an AE attack.

Methods

Patients

Patients were prospectively divided into 3 groups: patients with a confirmed bradykinin-mediated AE attack (known patient for HAE type I or II, or ACEi-related AE confirmed by the absence of recurrence after ACEi was stopped) consulting the French National Reference Centre for AE (CREAK) (group 1); patients with a mast cell-mediated AE attack defined by AE associated with acute urticaria (allergic AE), excluding patients with chronic spontaneous urticaria (group 2), and patients presenting with abdominal pains of known aetiology (control group or group 3). For group 1, patients were then divided into two subgroups (HAE and acquired AE related to ACEi). In group 3, the underlying causes of patients' abdominal pain were acute gastroenteritis, acute pancreatitis, acute colitis, faecaloma, gastritis, acute pyelonephritis, diverticulitis, and attack of Crohn's disease.

Exclusion criteria were: age <18 years, absence of written consent (refusal or impossible to obtain), impossible to take samples, absence of aetiological diagnosis, idiopathic AE, and HAE type III (because of a different physiopathological pattern). The same patient could be included several times if he/she had repeated AE attacks during the inclusion period. The clinical [intensity of the attack (assessed using a visual analogue scale), ongoing treatment, and progression] and paraclinical parameters were recorded at the time of inclusion and when each sample was taken. The clinician was allowed to select the treatment for each patient included. When necessary, CREAK was consulted for guidance as to patient management.

Table 1. Characteristics of the patients included

	Group 1	Group 2	Group 3
Patients, n	11	11	11
Men, n (%)	2 (18)	3 (27)	7 (64)
Median age, years (range)	62 (34–71)	59 (23–75)	66 (23–88)
Median duration of hospitalization, h (range)	34 (1–96)	45 (1–104)	125 (47–354)
Death, n	0	0	1
Specific treatments applied, with number of patients treated	Tranexamic acid: 2 C1INH concentrate: 3 Icatibant: 1 No treatment: 7	Antihistaminics: 11 Corticosteroids: 6 No treatment: 0	Analgesics Step 1: 11 Step 2: 6 Step 3: 4
Localization of oedema, with number of patients treated	ENT: 7 Extremities: 5 Abdominal: 2	ENT: 10 Extremities: 3 Torso: 2	NA
Median VAS H0 (range)	7 (4–10)	6 (5–10)	8 (6–10)

NA = Not applicable; VAS = visual analog scale.

All patients gave their written informed consent before inclusion in the study. The study protocol was examined and approved by the SUD EST V Ethics Committee (September 12, 2012) as well as authorized by the French National Agency for Drug safety (ANSM) on September 19, 2012.

Samples

Samples were collected from each patient several times during the study: during an attack and before any treatment was administered (H0), and then at 3, 6, 12, 24, and 48 h after the attack. An additional sample was collected 7 days after the attack (D7) when symptoms had resolved. For patients in group 1 registered at CREAK, samples were also taken upon inclusion in the study in the absence of AE symptoms.

Complement Assay

Assays to determine concentrations of C1INH and the C4 fraction of complement were performed on serum by nephelometry using an automated analyser (BNII; Siemens, France) and specific antisera (Siemens). C1INH activity was measured by spectrophotometry using the method described by Drouet et al. [10].

Assays were performed on samples taken at H0 and D7 after the attack. Samples (1 citrate and 1 dry tube without anticoagulant, 5 ml each) were sent directly to the laboratory at room temperature and were analysed immediately after centrifugation (15 min, 2,000 g, 20°C). The titration ranges used by the laboratory were as follows: weighted C1INH assay: 210–355 mg/l; C4 fraction: 100–380 mg/l, and C1INH activity: 17.2–27.4 U/ml.

D-Dimer Assays

Assays were performed at H0 and D7 after the attack. Samples were sent directly to the laboratory at room temperature (citrate tube, 5 ml) and analysed immediately using a standardized method (STA-Liatest D-DI; Stago, STA-R) after centrifugation (15 min, 2,000 g, 20°C). The lowest limit of detection was <0.27 mg/l, and the titration was linear up to 4 mg/l.

VE-Cadherin Assay, Marker of Vascular Stress

Circulating VE-cadherin was assayed at various time intervals, defined relative to the start of the attack (H0) and then at 3, 6, 12, 24, 48 h and D7 to establish the kinetics of changes to its concentration. The assay method was based on ELISA, as described in a previous study [11]. After sampling (dry tube without anticoagulant, 5 ml), patient serum was centrifuged at room temperature and stored at –80°C prior to performing the assay. No reference values had been defined, and the values reported were therefore only compared between the different patient groups.

Statistical Methods

Because of the small patient cohort, non-parametric statistical tests were used. The Mann-Whitney test was used for continuous quantitative variables and comparison of medians, and correlations were established based on Pearson's correlation coefficient. A p value <0.05 was taken as the threshold for statistical significance. Receiver operating characteristic (ROC) curves were created using XLSTAT 2015.1.01.

Results

Patients

Thirty-three individual cases were included from 1 January 2013 to 31 December 2014, corresponding to 28 patients: 11 attacks of bradykinin-mediated AE (7 type 1 HAE, 3 AE linked to an ACEi, and 1 case linked to an angiotensin 2 receptor antagonist, A2RA), 11 attacks of mast cell-mediated AE, and 11 patients in the control group (non-AE-related abdominal pains).

Patient characteristics are listed in table 1. The median time between the beginning of the attacks and the first sample (H0) was 1.56 h.

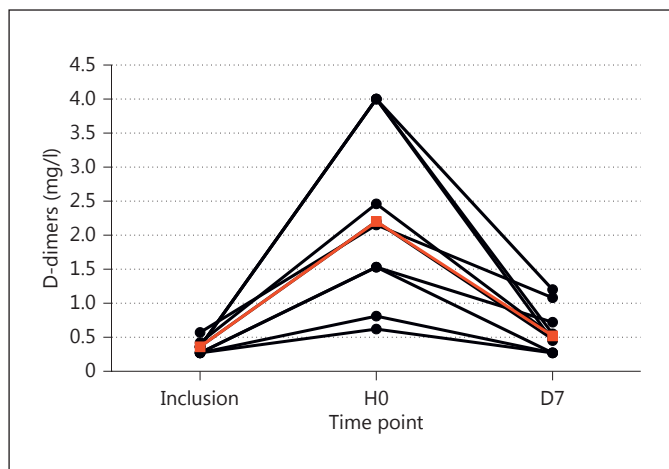


Fig. 1. Results of D-dimer assays for the 11 patients with bradykinin-mediated AE (group 1). The red curve represents the median for each time point (inclusion, H0 and D7). Color refers to the on-line version only.

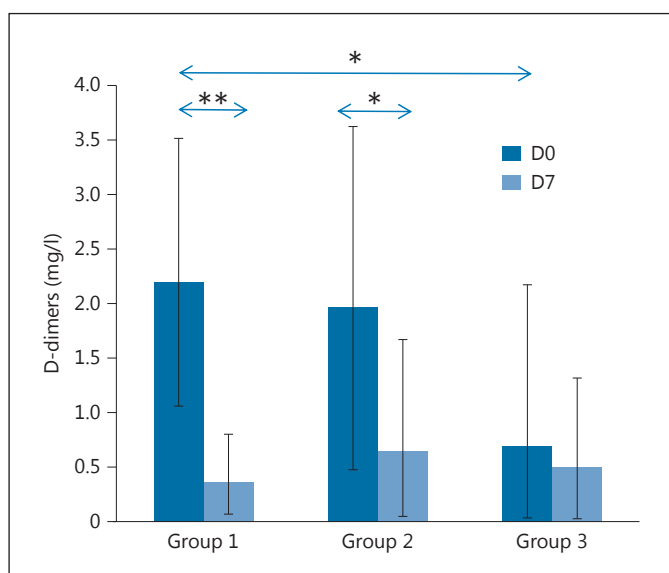


Fig. 2. D-dimer levels at H0 and D7 for the 3 groups: patients presenting with bradykinin-mediated AE (group 1), mast cell-mediated AE (group 2), or a non-bradykinin- and non-histamine-related abdominal pain of known aetiology (group 3). Median level and standard deviation bars are represented. * $p < 0.05$; ** $p < 10^{-3}$.

Within the control group, the diagnoses were as follows: acute gastroenteritis, acute pancreatitis (2 patients), acute colitis (2 patients, due to campylobacter and clostridium), faecaloma, gastritis, acute pyelonephritis (2 patients), diverticulitis, and attack of Crohn's disease.

D-Dimers

D-dimer levels were assayed upon inclusion, at H0 and D7 when clinical symptoms had resolved. In the group of bradykinin-mediated AE, a significantly higher level of D-dimers was noted during the attack compared to that at D7 (fig. 1; median of 2.2 vs. 0.52 mg/l, respectively; $p < 10^{-3}$) and compared to the time of inclusion (0.36 mg/l; $p < 10^{-3}$). No correlation was found between the intensity of the attack (assessed on an analogue visual scale) and the level of D-dimers ($p = 0.78$). No difference was found in the various attack types (ENT vs. peripheral vs. abdominal localization) in D-dimer levels. Sub-group analysis did not reveal any difference in D-dimer levels during the attack between patients with type 1 AE and AE related to ACEi or A2RA.

In the group of patients with mast cell-mediated AE (group 2), an increase in D-dimer level during the attack compared to that at D7 was also noted (1.97 vs. 0.65 mg/l; $p = 0.04$). The increase was similar in magnitude to the one observed for group 1 patients, and the difference between the median elevated D-dimer levels was non-significant when comparing group 1 and group 2 (2.2 vs. 1.97 mg/l; $p = 0.55$). In contrast, the level of D-dimers remained stable for patients in group 3 (0.69 vs. 0.52 mg/l; $p = 0.81$). It was also significantly higher during an attack for patients in group 1 than for those in group 3 (2.2 vs. 0.69 mg/l, respectively; $p = 0.01$; fig. 2). The difference between group 2 and group 3 at H0 did not reach statistical significance ($p = 0.25$).

Comparing group 1 (bradykinin-mediated AE) and group 3 (other causes of abdominal attacks), examination of the ROC curve indicates a 100% negative predictive value (NPV) for a threshold level of D-dimers of 0.62 mg/l. Moreover, the same NPV was observed for mast cell-mediated AE or group 2 (100%) compared to other causes of abdominal attacks (group 3).

C1INH Exploration

None of the parameters explored relating to C1INH showed any difference between H0 and the baseline levels for patients in group 1 presenting a C1INH deficit (7 patients). C1INH activity was low in subjects presenting type 1 HAE (median 2.6 U/ml during the attack vs. 2 U/ml at D7; $p = 0.61$), and weighted C1INH assays also returned low values without significant differences (37 vs. 42 mg/ml, respectively; $p = 0.40$), as did C4 assays (34.5 vs. 51 mg/ml, respectively; $p = 0.44$).

Similarly, the protein and activity levels of C1INH and the levels of C4 were stable in patients presenting drug-induced AE, as well as in patients in groups 2 and 3.

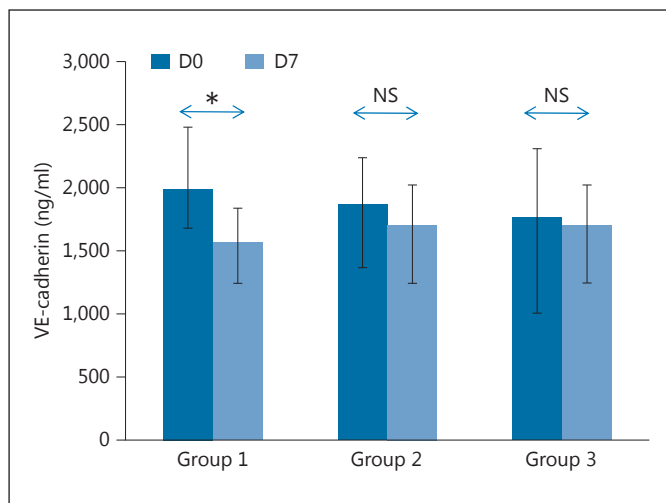


Fig. 3. Serum VE-cadherin levels at H0 and D7 in the 3 patient groups. Median level and standard deviation bars are represented. NS = Not significant. * $p < 0.05$; ** $p < 10^{-3}$.

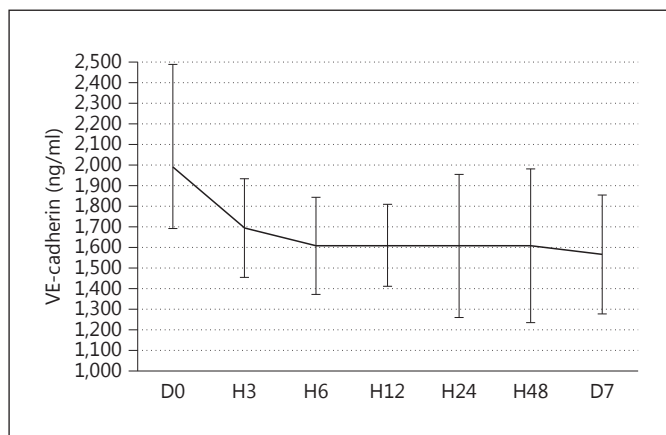


Fig. 4. Changes to median VE-cadherin levels over time during a bradykinin-mediated AE attack (group 1). Median level and standard deviation bars are represented.

VE-Cadherin

In group 1, the circulating VE-cadherin level was found to be higher during the attack (H0) than at D7 (1,990 vs. 1,566 ng/ml; $p = 0.01$; fig. 3). A study of the kinetics for this biomarker revealed a correlation between its levels and the intensity of the attack, as assessed by the doctor treating the patient ($\rho = 0.75$, $p = 0.04$) using a visual analogue scale. The kinetics for this marker were rapid, with levels increasing rapidly during the attack (H0) and normalizing as soon as clinical symptoms resolved

(fig. 4). No difference was found upon sub-group analysis (HAE vs. drug-induced AE).

The circulating levels of VE-cadherin were not different between patient groups during the attack (1,990, 1,869, and 1,764 ng/ml, respectively) or outside any attack (1,566, 1,705, and 1,703 ng/ml, respectively).

Discussion

This study revealed an increase in some serum markers during an AE attack. While a positive diagnosis of AE is generally based on clinical parameters, aetiological diagnosis sometimes remains difficult, in particular when bradykinin-mediated AE is associated with ENT or abdominal involvement.

An assay of D-dimers could prove useful in these cases. As shown previously [9, 12], C1INH is also implicated in the inhibition of activated factor XII, thrombin, and plasmin. In the absence of normal inhibition by C1INH, plasminogen activation results in plasmin formation [4], which is the principal enzyme that degrades stable fibrin lattices. This increased fibrinolytic activity could be evaluated in clinical practice by elevated D-dimers, resulting from fibrin degradation. D-dimers are usually used as biomarkers of extensive thrombosis. As previously noted by Cugno et al. [12] in a study of 28 patients, the levels of D-dimers measured during a bradykinin-mediated AE attack are significantly higher than the levels measured in control subjects. However, we noted a lower level of D-dimers during remission ($p < 10^{-3}$) than Cugno et al. [12] who found no advantage to using this marker based on its kinetics. The level of D-dimers was also increased in patients with urticaria and mast cell-mediated AE [13] and, thus, cannot distinguish the aetiological origin of AE. Probably due to a lack of power of this biomarker, we found no correlation between the level of D-dimers and the severity of the attack. A similar result was reported for AE associated with urticaria [14].

However, a threshold level of D-dimers can be defined based on the ROC curve. The 0.62-mg/ml threshold gave an NPV of 100% with the patient groups studied here. This threshold value can be used to exclude the diagnosis of an AE attack for patients presenting with abdominal pains (this is particularly useful with isolated abdominal attacks). A recent case reported suggests the usefulness of D-dimers for this purpose [15]. Nevertheless, the specificity of this criterion remained low (40%), because several other diseases also cause an increase in the level of this biomarker.

Exploration of C1INH and C4 levels did not find them to be useful biomarkers of AE attacks. The levels of these proteins or their activity did not vary in the group of patients with a C1INH deficit or in patients with acquired AE due to ACEi. Although in their study, Suffitti et al. [16] found a significantly lower C4 level during AE attacks than during remission, Cugno et al. [12] and Kadjaci et al. [17] reported similar results to ours. Therefore, exploration of C1INH and C4 in the context of an aetiological review of AE can be performed either during or after an attack, and its timing will not impact the diagnosis of C1INH deficit. As levels of these markers are not altered during mast cell-mediated AE attacks, it is possible to perform this assay during an attack in an emergency in case of doubt over the diagnosis between hereditary bradykinin-mediated AE (type 1 or 2) and mast cell-mediated AE.

The third and final marker explored in this study was a marker of endothelial stress: VE-cadherin. VE-cadherin is a transmembrane protein which plays an essential role in stabilizing adherens junctions between endothelial cells [18]. A recent publication [11] reported an increase in serum levels of this protein during an episode of bradykinin-mediated AE and an increased release of VE-cadherin upon exposure of an endothelial cell monolayer to bradykinin and kallikrein. Release of VE-cadherin from the cell membrane leads to an increase in vascular permeability due to intercellular disjunction. The results presented here confirm that circulating VE-cadherin levels increase during AE attacks in patients with bradykinin-mediated AE compared to basal levels (D7 and/or at time of inclusion); however, there was no significant difference between the VE-cadherin levels measured for the different patient groups (bradykinin-mediated AE, mast cell-mediated AE, and controls). The kinetics for VE-cadherin release were rapid, and the correlation between the intensity of the oedema and serum VE-cadherin levels indicates a probable physiopathological link between the cleavage of intercellular VE-cadherin, vascular permea-

bility, and the formation of oedema. The study of these kinetics showed that variations in circulating VE-cadherin are extremely rapid and can be used as a biomarker of progression for the same patient with bradykinin-mediated AE for whom the baseline levels of serum VE-cadherin are known.

The lack of difference between the 3 patient groups reflects the low specificity of this marker, which is an indicator of vascular stress. Vascular stress can occur during bradykinin-mediated AE, with significant histamine release or an intra-abdominal disorder. Although the results did not show any significant variations between the distinct aetiologies, this is the first time the kinetics of VE-cadherin release have been described during mast cell-induced AE. A larger patient cohort would perhaps show an increase in VE-cadherin reflecting increased vascular permeability in group 2 patients during the attack, as shown with bradykinin-mediated AE.

Conclusion

Exploration of how fibrinolysis is affected, particularly through D-dimer levels, constitutes a promising marker of AE attacks. A threshold level of D-dimers was shown to differentiate between an abdominal attack during bradykinin or mast cell-mediated AE and abdominal pain of other aetiologies. Thus, D-dimers could be useful in the emergency setting for patients with abdominal pain to determine an AE diagnosis versus other aetiologies. VE-cadherin, a protein marker of vascular stress, could not distinguish between mast cell-related AE and bradykinin-mediated AE, but presented a characteristic peak during an attack. Follow-up of these biological explorations and confirmation of our results by other teams should make it possible to use these markers in current practice for the differential diagnosis of these two physiopathological forms of AE, the identification of which determines the patients' therapeutic management.

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Retraction Statement

Paper by Deroux et al. Entitled "BIOBRAD Study: The Search for Biomarkers of Bradykinin-Mediated Angio-Oedema Attacks" [Int Arch Allergy Immunol. 2016;170(2):108–14, DOI: 10.1159/000446959]

We wish to retract the paper entitled "BIOBRAD Study: The Search for Biomarkers of Bradykinin-Mediated Angio-Oedema Attacks" [Int Arch Allergy Immunol. 2016;170(2):108–14, DOI: 10.1159/000446959] by Deroux et al.

At the beginning of the study, some procedural irregularities occurred during a quality approach, and this forces us, according to the French authority ANSM (Agence Nationale de Sécurité du Médicament et des Produits de Santé), to retract this article from the journal *International Archives of Allergy and Immunology*.

In this study, some patients whose consents were not conforming to the French rules were included. Therefore, their biological specimens could not be exploited. According to the promotor of the study, data from these patients must not be used and published anymore.

Alban Deroux, Laurence Bouillet, Grenoble