

# Different Rates of Autoreactivity in Patients With Recurrent Idiopathic Angioedema Associated or Not With Wheals

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## ■ Abstract

**Background:** The pathophysiology and triggers of idiopathic nonhistaminergic angioedema are unclear. This study aimed to assess autoreactivity in recurrent idiopathic angioedema associated or not with wheals.

**Methods:** The study population comprised 19 patients with recurrent idiopathic nonhistaminergic angioedema without wheals, 38 patients with angioedema and chronic urticaria (CU), and 52 patients with CU without angioedema. Twenty healthy individuals served as controls. Autoreactivity was evaluated *in vivo* using the autologous serum skin test (ASST) and *in vitro* by measuring serum-induced basophil histamine release (BHR).

**Results:** ASST results were negative in all patients with idiopathic angioedema without wheals and in healthy controls and positive in 29 of the 38 patients with angioedema and CU (76.3%) and in 26 of the 52 patients with CU without angioedema (50%) ( $P < .0001$  for both CU groups). BHR was negative in the healthy controls and positive in 2 of the 19 patients with idiopathic angioedema without wheals (10.5%), in 18 of the 38 patients with angioedema and CU (47.3%) ( $P < .0001$ ), and in 11 of the 52 patients with CU without angioedema (21.1%) ( $P < .03$ ).

**Conclusion:** The different rates of autoreactivity observed in patients with idiopathic nonhistaminergic angioedema without wheals and in patients with CU either with or without angioedema suggest that these disorders have a different pathophysiology. The failure to detect circulating vasoactive factors and histamine-releasing autoantibodies explains why H<sub>1</sub> antihistamines are scarcely effective in most patients with idiopathic angioedema without wheals. However, they represent the cornerstone of CU treatment.

**Key words:** Autologous serum skin test. Autoreactivity. Basophil histamine release. Idiopathic angioedema. Chronic urticaria.

## ■ Resumen

**Antecedentes:** Se desconocen la fisiopatología y los desencadenantes del angioedema idiopático no histaminérgico. El objetivo de este estudio fue evaluar la autorreactividad en el angioedema idiopático recurrente asociado o no a la presencia de habones.

**Métodos:** La población en estudio incluyó a 19 pacientes con angioedema idiopático no histaminérgico recurrente sin presencia de habones, a 38 pacientes con angioedema y urticaria crónica (UC), y a 52 pacientes con UC sin angioedema. Veinte individuos sanos sirvieron como controles. Se evaluó la autorreactividad *in vivo* mediante la prueba cutánea con suero autólogo (PCSA) e *in vitro* mediante la determinación de la liberación de histamina de basófilos (LHB) inducida por suero.

**Resultados:** Los resultados de la PCSA fueron negativos en todos los pacientes con angioedema idiopático sin presencia de habones y en los controles sanos, y positivos en 29 de los 38 pacientes con angioedema y UC (76,3%) y en 26 de los 52 pacientes con CU sin angioedema (50%) ( $p < 0,0001$  para ambos grupos con UC). Los resultados de la LHB fueron negativos en los controles sanos y positivos en 2 de los 19 pacientes con angioedema idiopático sin presencia de habones (10,5%), en 18 de los 38 pacientes con angioedema y UC (47,3%) ( $p < 0,0001$ ), y en 11 de los 52 pacientes con UC sin angioedema (21,1%) ( $p < 0,03$ ).

**Conclusión:** Los diferentes índices de autorreactividad que se observaron en pacientes con angioedema idiopático no histaminérgico sin presencia de habones y en pacientes con UC con o sin angioedema permiten suponer que estos trastornos tienen una fisiopatología distinta. El hecho de que no se detectaran factores vasoactivos circulantes y autoanticuerpos liberadores de histamina explica por qué los antihistamínicos H<sub>1</sub> resultan apenas eficaces en la mayoría de pacientes con angioedema idiopático sin presencia de habones y, sin embargo, representan la piedra angular del tratamiento de la UC.

**Palabras clave:** Prueba cutánea con suero autólogo; autorreactividad; liberación de histamina de basófilos; angioedema idiopático; urticaria crónica.

## Introduction

Angioedema is a self-limiting localized swelling that involves subcutaneous and submucosal tissues and is generally not associated with changes in skin color, burning sensation, or itchiness. It is thought to result from a local increase in the permeability of subcutaneous and submucosal capillaries and postcapillary venules in response to mediators such as histamine and bradykinin [1,2]. In most patients, angioedema occurs with wheals, is largely histaminergic, and responds to antiallergic drugs such as H<sub>1</sub> antihistamines and corticosteroids [3-5]. The most common form is allergic angioedema, which is generally due to allergy to food, drugs, and insect venom [3]. Immunoglobulin (Ig) E-mediated mast cell degranulation with subsequent histamine release is considered to underlie the disorder in most cases. However, the pathophysiology of recurrent angioedema with urticaria may also be linked to an autoimmune process leading to mast cell degranulation. Chronic spontaneous urticaria (CU), which is associated with angioedema in about 30% of cases, is now recognized as an autoimmune disease associated with histamine-releasing autoantibodies in a consistent proportion of patients [6]. Substantial advances in our understanding of the pathogenesis of this disease have been made during the last 2 decades, following the observation by Grattan et al [7] that intradermal injection of autologous serum (autologous serum skin test [ASST]) caused a wheal-and-flare reaction in about 50% of CU patients. The main factors inducing skin reactivity to autologous serum were subsequently identified as autoantibodies directed against the  $\alpha$  subunit of the high affinity IgE receptor (Fc $\epsilon$ RI) or against IgE [8,9]. These findings led to the interpretation of CU as an autoimmune disorder produced by histamine-releasing autoantibodies in about 40% of patients. In contrast to angioedema occurring in the context of an allergic reaction or CU, the pathophysiology of recurrent idiopathic angioedema without urticaria remains unclear. Although bradykinin is known to play a major role in the pathophysiology of angioedema caused by hereditary or acquired C1-inhibitor deficiency and treatment with angiotensin-converting enzyme inhibitors, the mediators involved in idiopathic nonhistaminergic angioedema and related factors acting as triggers or enhancers of vascular permeability remain unclear [2,10,11].

The aim of this study was to assess autoreactivity in patients with idiopathic recurrent angioedema associated or not with urticaria. Autoreactivity was evaluated *in vivo* using the ASST and *in vitro* by measuring serum-induced basophil histamine release (BHR).

## Patients and Methods

The study population comprised 19 patients with recurrent idiopathic angioedema without wheals (13 males and 6 females; median age, 45 [range, 27-78] years), 38 patients with angioedema and CU (8 males and 30 females; median age, 40.5 [range, 14-76] years), and 52 patients with CU without angioedema (16 males and 36 females; median age,

37.5 [range, 18-81] years), all of whom were evaluated at the allergy center of Ospedale Maggiore Policlinico in Milano, Italy. Twenty healthy individuals (11 males and 9 females; median age, 40.5 [range, 26-65] years) were selected as controls. CU was diagnosed on the basis of the recurrence of spontaneous wheals for more than 6 weeks. Physical urticaria and other possible causes of urticaria (ie, food and drug allergy and parasitosis) were ruled out after appropriate investigations. Only patients with chronic spontaneous urticaria were selected for the study, whereas patients with physical urticaria and urticaria vasculitis were excluded.

At the first visit, based on patients' recent history and according to the number of wheals and degree of pruritus present, disease activity was estimated following the urticaria activity score (UAS), as recommended in the recent guidelines of the European Academy of Allergy and Clinical Immunology [12]. According to the UAS, urticaria was classified as mild (score 1), moderate (score 2), or severe (score 3). All patients had active urticaria with or without angioedema at the time of the study.

Patients with idiopathic angioedema without wheals reported a history of recurrent angioedema involving different sites and organs (mostly affecting the face [including lips and eyelids], hands, feet, and genital area) that was not accompanied by urticaria and was not triggered by identified external agents including drugs, foods, or hymenoptera venom. None of the patients had a family history of angioedema or was taking angiotensin-converting enzyme inhibitors. Functional C1-inhibitor levels, measured using a commercial reagent kit (Immuno), and antigenic C1-inhibitor levels, assayed using radial immunodiffusion (Dade Behring), were normal in all cases, as were serum levels of the complement fractions C3 and C4. In all patients, treatment with H<sub>1</sub> antihistamines at the licensed doses had failed to prevent angioedema episodes.

The intradermal ASST was performed with 0.05 mL of fresh autologous serum at least 5 days after stopping antihistamine therapy (cetirizine, levocetirizine, loratadine, desloratadine, or ebastine in all cases) according to the method of Sabroe et al [13], with reading of the wheal-and-flare reaction at 30 minutes. Intradermal injection of saline solution (0.9% wt/vol) was performed as a negative control and skin prick test with 10 mg/mL of histamine as a positive control. Patients showing a red wheal with a diameter at least 1.5 mm greater than that of the control saline solution were considered positive. At the time of blood sampling, none of the patients had associated chronic inflammatory disorders, hepatitis C, or a history of recent acute infectious disease. All participants gave their informed consent to undergo ASST and peripheral blood collection for *in vitro* assays.

### Basophil Histamine Release Assay

Leukocyte suspensions from healthy blood donors were prepared by dextran sedimentation of peripheral venous blood anticoagulated with 0.01 M EDTA and mixed with 6% dextran in saline solution (Plander 70, Fresenius Kabi Italia) and 30 mM dextrose (Sigma Chemicals). The cells were allowed to settle for 60-90 minutes at room temperature, the leukocyte-rich plasma was aspirated and centrifuged at 300g for 15

minutes at 4°C, and the cell button was washed twice in Tyrode buffer (pH, 7.4) containing (mM) 140 NaCl, 5.5 dextrose, 2.7 KCl, 0.36 NaH<sub>2</sub>PO<sub>4</sub>, and 12 NaHCO<sub>3</sub>. Leukocytes (with about 7 × 10<sup>4</sup> basophils) were resuspended in 100 µL of Tyrode buffer with 1.8 mM CaCl<sub>2</sub> and 0.5 mM MgCl<sub>2</sub> and incubated with 100 µL of the serum under examination, making a final volume of 200 µL. After incubation for 40 minutes at 37°C, the reaction was stopped by addition of 800 µL of ice-cold buffer solution and centrifugation at 1000g for 10 minutes at 4°C. The supernatants were then aspirated, mixed with an equal volume of 6% HClO<sub>4</sub> and centrifuged again at 1000g for 10 minutes at 4°C. The histamine concentration in the supernatants was measured using an automated fluorometric method [14]. Spontaneous histamine release was evaluated by measuring the histamine concentration in the supernatant of nonstimulated cells incubated for 40 minutes at 37°C. Total histamine content was obtained by adding 100 µL of 6% HClO<sub>4</sub> to 100 µL of cell suspension. Net histamine release was calculated as the percentage of total histamine content after subtraction of spontaneous release. A 5% release cutoff value was used, as previously described [15]. Sera were tested with leukocyte suspensions from a normal donor whose basophils were previously shown to release 30% of total histamine content on challenge with an optimal dose of goat polyclonal antihuman IgE (Sigma Chemicals; 10 µg/mL). In order to detect the presence of functionally active anti-FcεRI autoantibodies, basophil histamine release was also evaluated after stripping membrane-bound IgE with 10 mmol/L of lactic acid (pH, 3.9; incubation for 3.5 minutes at room temperature), a procedure that allows dissociation of FcεRI-bound IgE without damaging basophil functional activity [16]. One assay per serum was performed to detect histamine-releasing activity. The histamine release assay was carried out using the same sera as for ASST.

### Statistical Analysis

Results were expressed as median and range. Proportions were compared using the Fisher exact test. Differences between patient groups were assessed using the Kruskal-Wallis test and Dunn test. The correlation with the UAS was assessed using the Spearman rank test. *P* values <.05 were considered significant.

### Results

No differences were found between the 3 patient groups for median age, whereas a significant difference was

found for sex distribution, namely, a predominance of males among patients with idiopathic angioedema without wheals (68.4%) and a minority of males among patients with angioedema and CU (21%, *P*=.001) and among patients with CU without angioedema (30.7%, *P*=.018) (Table 1). The ASST result was negative in all healthy controls and in patients with idiopathic nonhistaminergic angioedema without urticaria; conversely, it was positive in 29 out of 38 patients with angioedema and CU (76.3%) and in 26 out of 52 patients with CU without angioedema (50%). ASST positivity was significantly associated with CU with or without angioedema in comparison to patients with idiopathic angioedema without wheals (*P*<.0001 for both patient groups, relative risk, 3.22 for CU with angioedema and 1.76 for CU without angioedema); furthermore, percentage positivity was higher in patients with CU and angioedema than in patients with CU without angioedema (*P*=.015). BHR was positive in 2 out of 19 patients with idiopathic angioedema without wheals (10.5%), in 18 out of 38 patients with angioedema and CU (47.3%) (*P*<.0001 vs healthy controls, relative risk 2.0; *P*=.007 vs angioedema patients), and in 11 patients out of 52 with CU without angioedema (21.1%) (*P*<.03 vs healthy controls; relative risk, 1.48) (Table 2). The results of histamine release are reported in the Figure and show a significant difference between the 4 groups (*P*=.0001). BHR was positive only after stripping of basophil membrane-bound IgE with lactic acid in 1 patient with angioedema without wheals (out of 2 positive), in 1 patient with angioedema and CU (out of 18 positive), and in 3 patients with CU without angioedema (out of 11 positive). No correlations were found between BHR and CU severity score in patients

Table 1. Demographic Characteristics

Patient Category	Number	Male/Female Ratio	<i>P</i> Value <sup>a</sup>	Median Age (Range), y
Healthy controls	20	11/9		40.5 (26-65)
Angioedema without urticaria	19	13/6		45 (27-78)
Angioedema with chronic urticaria	38	8/30	.001	40.5 (14-76)
Chronic urticaria without angioedema	52	16/36	.018	37.5 (18-81)

<sup>a</sup>Fisher exact test compared to patients with angioedema without urticaria

Table 2. Positivity of Autologous Serum Skin Test and Basophil Histamine Release Assay

Patient Category	ASST-Positive,%	<i>P</i> Value <sup>a</sup>	BHR-Positive,%	<i>P</i> Value <sup>a</sup>
Healthy controls	0/20		0/20 (0)	
Angioedema without urticaria	0/19 (0)	NS	2/19 (10.5)	NS
Angioedema with chronic urticaria	29/38 (76.3)	<.001	18/38 (47.3)	.0001
Chronic urticaria without angioedema	26/52 (50)	<.001	11/52 (21.1)	.03

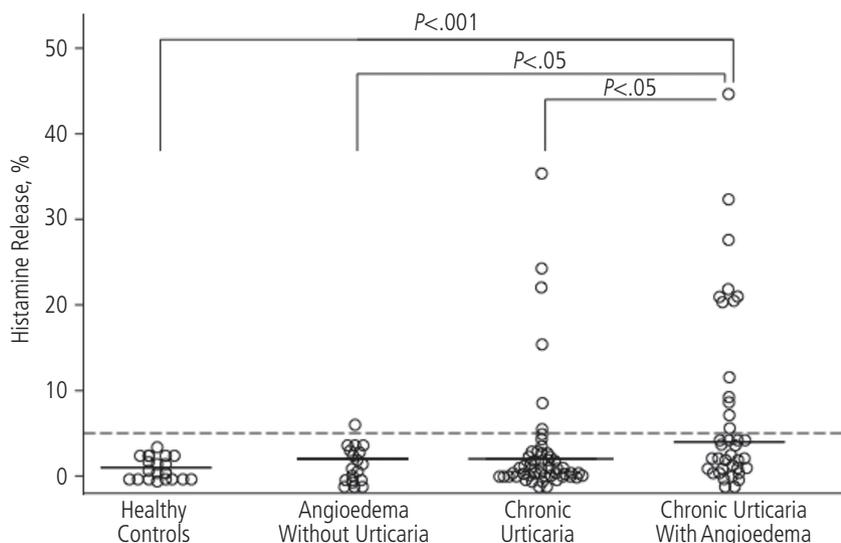
Abbreviations: ASST, autologous serum skin test; BHR, basophil histamine release; NS, nonsignificant.

<sup>a</sup>Fisher exact test compared with healthy controls.

with CU and angioedema or in patients with CU without angioedema.

## Discussion

The results of this study indicate that patients with idiopathic angioedema without wheals do not react to intradermal injection of autologous serum and do not show significant circulating histamine-releasing activity, unlike patients with angioedema and CU, who often show autoreactivity. In fact, CU is associated with the presence of circulating histamine-releasing factors, as assessed by ASST and BHR, independently of the coexistence of angioedema. Skin reactivity to intradermal injection of autologous serum in about 50% of patients with CU was described in 1986 by Grattan et al [7] and considered indicative of circulating vasoactive factors that could be relevant to the pathogenesis of the disease. In subsequent years, histamine-releasing autoantibodies targeting the high-affinity IgE receptor or IgE were identified in patients with CU [8,9], and ASST was proposed as a screening test for functional autoantibodies in CU patients [13]. Nowadays, ASST is considered more a test for autoreactivity than a specific test for histamine-releasing autoantibodies, as it has been shown to have moderate specificity as a marker for functional autoantibodies targeting FcεRI or IgE, but a high negative predictive value (92.8%) for patients with or without CU [17]. According to the expert panel of the European Academy of Allergology and Clinical Immunology, a negative ASST result is a useful surrogate marker of the absence of circulating histamine-releasing autoantibodies [17]. Conversely, BHR has been used as a confirmatory test showing the presence of functionally active histamine-releasing autoantibodies [18], since a routine in vitro assay able to detect circulating and functionally active anti-FcεRIα and/or anti-IgE autoantibodies is still lacking. Negative ASST results in patients with idiopathic angioedema without wheals enable us to rule out the presence of histamine-releasing autoantibodies in the vast majority of patients. In fact, we were able to detect weak serum histamine-releasing activity just above the cutoff value in 2 out of 19 patients with idiopathic angioedema without wheals, indicating that small amounts of histamine-releasing autoantibodies may occasionally be present in this group. In one patient, histamine-releasing activity was detected using basophils bearing membrane IgE, indicating the presence of anti-IgE autoantibodies, whereas in the other patient, histamine release was observed only after stripping basophil-bound IgE, thus suggesting the presence of anti-FcεRI autoantibodies. Consistent with previous observations, patients with CU either with or without angioedema showed frequent autoreactivity, since ASST was positive in 76.3% and 50% of cases, respectively, suggesting the presence of circulating



**Figure.** Results of serum-induced basophil histamine release in healthy controls and in patients with idiopathic angioedema without urticaria, chronic urticaria without angioedema, and chronic urticaria with angioedema. Individual values are reported. Horizontal bars are median values. The horizontal dotted line represents the cutoff value.

vasoactive factors and/or histamine-releasing autoantibodies in at least half of the patients [7,17]. It is interesting to note that the percentage of positive ASST results was higher in patients with CU and angioedema than in patients with CU without angioedema, thus confirming the observation by Nettis et al [19], who noted that CU patients with angioedema were more frequently ASST-positive than those without angioedema. Accordingly, patients with a positive basophil activation test result showed a higher prevalence of other autoantibodies and more severe urticaria and were more likely to have angioedema [20]. However, this has not been a consistent finding in other studies [21,22]. A significant difference between the 3 patient groups was also found for BHR: patients with angioedema and CU had the strongest serum histamine-releasing activity. Therefore, the overall impression is that autoreactivity is more common and stronger in patients with angioedema and CU than in patients with CU without angioedema. It is noteworthy that the percentage of positive BHR results was lower than the percentage of positive ASST results in both CU groups, indicating lower sensitivity for the former than for the latter, as found in a previous study by our group [15] and confirmed by Platzer et al [23]. This finding may be explained, at least in part, by the characteristics of the basophils used in the assay, such as releasability, density of membrane IgE, and availability of free FcεRI receptors; in fact, percentage positivity increases if basophils from different donors or human mast cells are used [24].

In conclusion, negative ASST findings demonstrate the lack of circulating vasoactive factors and histamine-releasing autoantibodies in most patients with idiopathic nonhistaminergic angioedema without wheals, in contrast to patients with CU either with or without angioedema. Furthermore, the predominance of males among patients with idiopathic nonhistaminergic angioedema does not support an autoimmune origin for this disorder, whereas the predominance

of women among patients with CU is consistent with the known predilection of autoimmune disorders in females [25]. The different rates of autoreactivity, as assessed by ASST and BHR, provide an explanation for the different efficacy of H<sub>1</sub> antihistamines. In fact, H<sub>1</sub> antihistamines are scarcely effective in most patients with idiopathic angioedema without wheals, whereas they represent the cornerstone of CU treatment. Assessment of autoreactivity by ASST, BHR, or both may help to predict the response to H<sub>1</sub> antihistamines in patients presenting with recurrent angioedema.

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