Original Article

A Comparative Study of Sex Distribution, Autoimmunity, Blood, and Inflammatory Parameters in Chronic Spontaneous Urticaria with Angioedema and Chronic Histaminergic Angioedema

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What is already known about this topic? Chronic histaminergic angioedema (CHA) is classified as a form of nonhereditary and mast cell–mediated recurrent angioedema dependent on mast cells. However, when dealing with the nosology of urticarial disorders, it is typically considered as part of the definition of chronic spontaneous urticaria (CSU). CHA does share clinical features and approaches to treatment with CSU, which adds to the confusion.

What does this article add to our knowledge? This work compares for the first time CHA and CSU (considering those patients with hives and angioedema) side by side, observing some remarkable differences in gender distribution, basophil number, and antibodies against the IgE receptor.

How does this study impact current management guidelines? Until there are further studies that either support or refute what we propose, CHA and CSU should not automatically be considered the same disorder.

BACKGROUND: Recurrent idiopathic histaminergic angioedema is currently classified as a subtype of angioedema, as well as a subtype of chronic spontaneous urticaria (CSU), based on the fact that both are mast cell-mediated and respond to the same treatments.

OBJECTIVE: In the present work, we sought to verify whether chronic histaminergic angioedema (CHA) is an entity distinct from CSU or represents a CSU subtype that lacks hives.

METHODS: We performed a prospective study comparing 68 CHA patients, angioedema without hives, with 63 CSU patients, with hives and angioedema, from whom we collected demographic and clinical data, as well as blood and serum markers.

RESULTS: We found key pathogenic features that differentiate CHA from CSU: gender distribution, basophil number, and antibodies against the IgE receptor. The male/female ratio in CHA was 0.78, whereas in CSU it was 0.36 ($P = .0466$). Basopenia was more often seen in CSU (n = 13 [20%]) than in CHA (n = 5 [7%]). Finally, 31.15% of CSU sera induced basophil activation, whereas no CHA sera were able to activate normal basophils. By contrast, nonspecific inflammation or immune markers, for example, erythrocyte sedimentation rate,

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Abbreviations used
AE- Angioedema
ANA- Antinuclear antibodies
BAT- Basophil activation test
CASP-8- Caspase-8
CHA- Chronic histaminergic angioedema
CRP- C-reactive protein
CSU- Chronic spontaneous urticaria
ESR- Erythrocyte sedimentation rate
ISCIII- Instituto de Salud Carlos III
NSAIDs- Nonsteroidal anti-inflammatory drugs
OPG- Osteoprotegerin
OSM- Oncostatin-M
TGF-α- Transforming growth factor alpha
TNF-α- Tumor necrosis factor alpha
VEGF- Vascular endothelial growth factor

C-reactive protein, or IgG antithyroid antibodies, were very similar between both groups. IgE anti−IL-24 could not be assessed because a control population did not differ from CSU.

CONCLUSIONS: Inclusion of CHA as part of the spectrum of CSU is an assumption not evidence-based, and when studied separately, important differences were observed. Until there is further evidence, CHA and CSU should not necessarily be considered the same disorder, and it is our opinion that review articles and guidelines should reflect that possibility. **(J Allergy Clin Immunol Pract 2021;9:2284-92)**

Key words: *Angioedema; Urticaria; Histaminergic angioedema; Chronic urticaria; Basophils*

Chronic histaminergic angioedema (CHA) belongs to a "nobody's land," *terra nullius*. When dealing with angioedema (AE), it is classified as a nonhereditary and mast cell−mediated subtype of AE,[1,2] and when dealing with urticaria, it is included in the definition of CSU.[3] AE is defined as a self-limited swelling of the deep dermis and/or subcutaneous tissue as well as the submucosal surface due to the action of vasoactive mediators.[4] There are 2 types depending on the particular mediator involved, namely, bradykinin-mediated AE or histamine-mediated AE (also known as mast cell−mediated AE). CHA, as described in the last European guideline for AE,[1] is classified specifically as idiopathic histaminergic acquired AE and can be diagnosed when recurrent AE without hives with no underlying cause is found, if it responds to either antihistamines, corticosteroids, adrenaline, or most recently, omalizumab.[5] CHA is also typically described as a subtype of chronic spontaneous urticaria (CSU).[2,3] CSU is associated with AE accompanying the urticaria in approximately 40% to 50% of patients,[6] a similar proportion to those presenting with urticaria alone. A remaining 10% to 20% are described as urticaria without the wheals,[7] which we address in this article under the name CHA. Implicit in this description is that the pathogeneses of the various subtypes are similar, if not identical. That is clearly true for those patients with urticaria, whether AE is present or absent.[8] However, there are far fewer data involving patients with CHA, and our purpose is to explore objectively how one distinguishes a subtype of CSU from a separate disorder. Being mast cell dependent or responsive to antihistamines or omalizumab are insufficient criteria to assume that they have to be the same. For example, urticarial disorders such as cold urticaria and dermatographism fulfill these criteria and are clearly different disorders. In the present work, we sought to distinguish these 2 possibilities.

For that purpose, we compared clinical features, demographic distribution, and several biological markers of 2 similar populations; one comprised patients suffering from CSU with accompanying AE (not only hives) and the other comprised patients suffering from isolated histaminergic AE, identified as CHA in our article but often referred to as CSU with only AE despite the lack of evidence. We also assessed autoimmunity and basophil parameters in both populations, because autoimmunity[9,10] and basopenia[11] are key features of CSU.

MATERIAL AND METHODS

Patients and clinical data

We performed a prospective study comparing 68 patients with CHA and 63 CSU patients with hives and AE recruited from 6 hospitals across Spain. Throughout 3 years, patients were prospectively included when attending their corresponding center. At the visit time, we collected all the demographic and clinical features, AE characteristics, treatment, blood biomarkers, and serum, which was stored at −80 °C.

All patients were older than 18 and gave signed informed consent to participate in the study. Ethics approval was obtained in each of the collaborating centers, and the study followed Good Clinical Practice guidelines and the Declaration of Helsinki.

The inclusion criteria for CSU required the daily or almost daily presence of hives for more than 6 weeks and AE. The exclusion criteria were the presence of only hives without AE.

To differentiate CHA from bradykinin-mediated AE or other inducible AE, we established that the inclusion criteria to fulfill the diagnosis of CHA were the presence of recurrent AE that responds to treatment with antihistamines, corticosteroids, adrenaline, or omalizumab. The exclusion criteria were the presence of bradykinergic AE, angiotensin-converting enzyme-inhibitor AE, delayed pressure urticaria/AE, vibratory AE, or AE induced by nonsteroidal anti-inflammatory drugs (NSAIDs). All patients had normal C1INH, C1q, C3 and C4 protein levels, and normal C1INH activity.

Blood biomarkers

Blood count and blood/serologic markers (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], D-dimer, thyroid-stimulating hormone, free T4, total IgE levels, antinuclear antibody [ANA] titer, and thyroid antibodies) were obtained in each clinical laboratory center.

Basophil activation test

To assess the ability of patients' serum to activate normal basophils, basophils from healthy donors were incubated with sera from patients, as previously described.[12] Briefly, 50 μL of buffy coat blood resuspended in activation buffer (containing 2 ng/mL of IL-3) was stimulated with 50 μL of patients' sera. A mouse antihuman IgE (Clone G7-26; BD Bioscience) was used as positive control. After 30 minutes at 37 °C, cells were washed and stained for 30 minutes at 4 °C with CD63 PE, CD123 PerCp-Cy5.5, HLA-DR PE-Cy7, and CD203c APC (all from BD Bioscience). Red blood cell lysis was performed next for 10 minutes at room temperature, with BD Pharm Lyse (BD Bioscience). Finally, samples were washed twice and acquired in a FACS Canto II flow cytometer (BD Bioscience).

TABLE I. Demographic and clinical features from the patients

	CHA (n = 68)	CSU (n = 63)	*P* value
Gender (male), N (%)	30 (44.12)	17 (26.98)	**.0466**
Male/female ratio	0.78	0.36	
Age (y), mean (range)	53.57 (20.2-85.4)	47.96 (20.7-76.9)	**.0359**
Atopy (yes), N (%)	11 (16.18)	15 (23.81)	.2842
Comorbidities, N (%)			
Diabetes	7 (10.29)	5 (7.94)	.7656
HBP	16 (23.53)	8 (12.70)	.1203
Thyroiditis	3 (4.41)	8 (12.70)	.1177
COPD	1 (1.47)	1 (1.59)	>.999
Other AID*	1 (1.47)	4 (6.35)	.1948
Family history†, N (%)			
Yes	19 (27.94)	22 (34.92)	.4524
AE	7 (10.29)	1 (1.59)	.0636
CSU‡	1 (1.47)	9 (14.29)	**.0070**
Atopy	10 (14.71)	10 (15.87)	>.999
AID	3 (4.41)	5 (7.94)	.4803

AE, Angioedema; *AID*, autoimmune disease; *CHA*, chronic histaminergic angioedema; *COPD*, chronic obstructive pulmonary disease; *CSU*, chronic spontaneous urticaria; *HBP*, high blood pressure.
Bold values indicate statistical significance ($P < .05$).
*Other AID: hypothyroidism, Addison disease, and vitiligo were reported.
†Family history of AE, CSU, atopy, or autoimmune disease.
‡Includes CSU with AE and CSU without AE.

TABLE II. Angioedema: evolution and episodes

	CHA (n = 68)	CSU (n = 63)	*P* value
AE time of evolution (y), median (range)	3 (0-60)	2 (0-44)	.4857
AE episode duration (h), median (range)	27 (2-108)	18 (2-181)	.2714
Relationship with menstruation (only women) (yes), N (%)	4 (10.53)	5 (10.87)	>.999
Takes oral contraceptives (only women) (yes), N (%)	2 (5.26)	5 (10.87)	.4487
NSAID intolerance (yes), N (%)	10 (14.71)	18 (28.57)	.0586
Affected areas, N (%)			
Lips	54 (79.41)	60 (95.24)	**.0086**
Eyelids	35 (51.47)	47 (74.60)	**.0071**
Tongue	40 (58.82)	18 (29.03)	**.0008**
Uvula	15 (22.06)	13 (20.63)	>.999
Extremities	21 (30.88)	24 (38.10)	.4622
Genitalia	10 (14.71)	4 (6.35)	.1602

AE, Angioedema; *CHA*, chronic histaminergic angioedema; *CSU*, chronic spontaneous urticaria; *NSAID*, nonsteroidal anti-inflammatory drug.
Bold values indicate statistical significance ($P < .05$).

Doublets and dead cells were eliminated based on forward and side scatter parameters. Basophil populations were selected as CD123+ HLA-DR- cells, and the gate limit for CD63 positivity was established using the positive control tube. The basophil activation test (BAT) was considered positive when more than 5% of the total basophils were CD63-positive. The value of the 95th percentile of CD63+ cells induced by control sera was established based on previously published data.[13]

Targeted proteomics

Ninety-two inflammation-related proteins (Inflammation panel, available on www.olink.com) were measured in serum samples using a proximity extension immunoassay (Olink Proteomics, Uppsala, Sweden). The methodology has been described elsewhere.[14,15] Results are reported as the normalized protein expression value, an arbitrary unit that is in log-2 scale.

IgE anti–IL-24 ELISA

Nunc MaxiSorp flat-bottom 96-well plates (Thermofisher) were coated with recombinant human IL-24 (R&D Systems), 2 μg/mL per well, overnight at 4 °C. The wells were washed with phosphate buffered saline with 0.05% Tween 20 (Merck-Millipore). They were blocked with SuperBlock Blocking Buffer (Thermofisher). Then 100 μL of serum was added per well, and the plate was incubated for 2 hours. Afterward, the wells were washed and incubated with a biotin-conjugated goat antihuman IgE antibody (Thermofisher) for 1 hour. Pierce High Sensitivity Streptavidin-HRP, 1-Step Ultra TMB-ELISA Substrate Solution, and Stop Solution for TMB substrates (all from Thermofisher) were used for the detection of the bound IgE. Plates were read at 450 nm.

In addition to patient samples, we tested serum samples from control individuals, including nonatopic and atopic individuals

TABLE III. Hematological/serological parameters

	CHA (n = 67)*	CSU (n = 63)	*P* value
Erythrocytes ($\times 10^6$/mm^3), mean (SD)	4.79 (0.42)	4.93 (0.53)	.1519
Platelets ($\times 10^3$/mm^3), mean (SD)	242.50 (43.96)	260.60 (69.85)	.1895
MPV (fL), mean (SD)	10.47 (1.07)	10.31 (1.13)	.2811
PDW† (%), mean (SD)	15.29 (6.86)	13.52 (2.41)	.4182
Leukocytes ($\times 10^3$/mm^3), mean (SD)	6.83 (1.97)	7.49 (1.98)	.0673
Neutrophils ($\times 10^3$/mm^3), mean (SD)	4.06 (1.52)	4.92 (1.82)	**.0051**
Lymphocytes ($\times 10^3$/mm^3), mean (SD)	2.04 (0.72)	2.21 (0.83)	.2958
Monocytes ($\times 10^3$/mm^3), mean (SD)	0.51 (0.17)	0.48 (0.16)	.2759
Eosinophils ($\times 10^3$/mm^3), mean (SD)	0.17 (0.14)	0.15 (0.14)	.1856
Basophils, mean (SD)$\times 10^3$/mm^3	0.045 (0.03)	0.032 (0.03)	**.0028**
%	0.69 (0.36)	0.42 (0.30)	**<.0001**
ESR (mm), mean (SD)	13.27 (10.80)	12.07 (11.50)	.2628
CRP (mg/dL), mean (SD)	0.48 (1.13)	0.50 (0.77)	.1146
D-dimer (ng/mL), mean (SD)	491.30 (1318)	560.70 (989.90)	.2895
TSH (mUI/L), mean (SD)	1.81 (1.01)	2.62 (6.88)	.9067
Free T4 (ng/dL), mean (SD)	1.23 (0.28)	1.19 (0.21)	.6373
Total IgE (kU/L), mean (SD)	166.7 (222.80)	141.3 (236.30)	**.0330**

CHA, Chronic histaminergic angioedema; *CRP*, C-reactive protein; *CSU*, chronic spontaneous urticaria; *ESR*, erythrocyte sedimentation rate; *MPV*, mean platelet volume; *PDW*, platelet distribution width; *SD*, standard deviation; *T4*, thyroxine; *TSH*, thyroid-stimulating hormone.
Bold values indicate statistical significance ($P < .05$).
*From 1 patient, results were not obtained.
†PDW was only available in 2 centers: N for CHA = 18 and N for CSU = 30.

(N = 51; mean age: 43.50, range: 16.53-68.04; male individuals: 18, 35.29%). All samples were tested in at least 3 different assays.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 (GraphPad Software, San Diego, Calif). Qualitative variables are reported as the total number and a percentage and were compared using the Fisher exact test. Quantitative variables are reported as mean with standard deviation or range. They were compared using the Student *t* test with Welch's correction (normally distributed) or the Mann-Whitney *U* test (not normally distributed). For targeted proteomics, Mann-Whitney tests were performed. To test for normality distribution, we used the D'Agostino-Pearson test. Values were considered significant with $P < .05$.

RESULTS

Our study aimed to compare patients with CHA, that is recurrent isolated AE that responds to antihistamines, corticosteroids, adrenaline, or omalizumab, with patients with CSU who presented clinically with hives and AE and who also respond to the same treatments. The main differences observed between groups include age and sex differences, AE location, absence of anti-IgE or anti-FcεRI in CHA, and different levels of several serum proteins.

CHA and CSU demographics

As observed in Table I, there was a significantly different gender distribution between the 2 groups: the proportion of women was significantly higher in CSU (male/female ratio in CHA was 0.78, whereas in CSU it was 0.36, $P = .0466$). Likewise, mean age was significantly higher in CHA (53.6 years) than in CSU (47.6 years, $P = .0244$), whereas the rest of the demographic parameters were similar between both conditions.

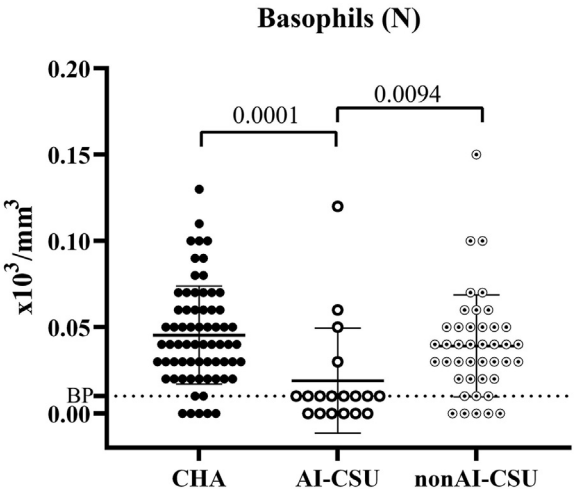

FIGURE 1. Basophil numbers in the CHA and CSU groups subdivided into autoimmune (AI-CSU, with the positive BAT result) or non-autoimmune (nonAI-CSU, with the negative BAT result). Groups compared with Kruskal-Wallis with Dunn's *post hoc* test, *P* values shown in the figure. *BAT*, Basophil activation test; *BP*, basopenia cutoff value at 0.1 $\times 10^3$ basophils/mm^3; *CHA*, chronic histaminergic angioedema; *CSU*, chronic spontaneous urticaria; *AI-CSU*, autoimmune-chronic spontaneous urticaria.

Family history

Patients were specifically questioned about their family background regarding AE, CSU, atopy, and autoimmune diseases. When considering all the variables together, no significant differences were observed between the groups (Table I). However, patients suffering from CHA tended to report more AE in

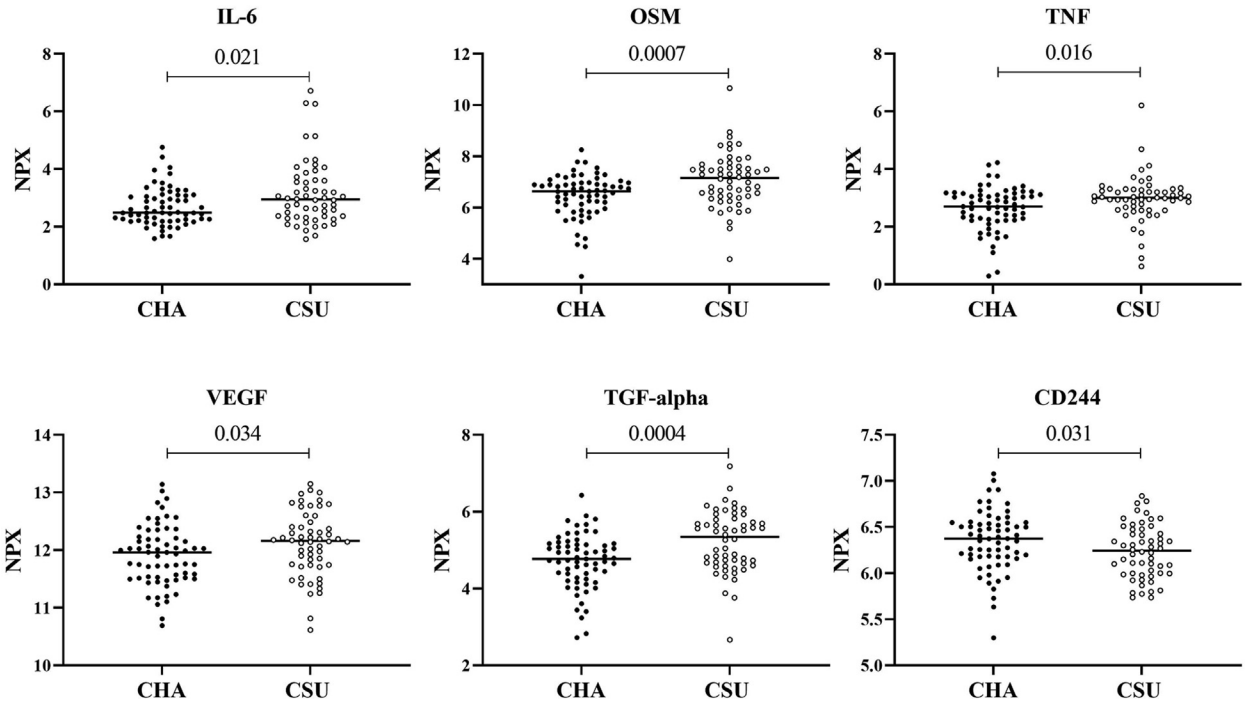

FIGURE 2. NPX values for IL-6, OSM, TNF-α, VEGF, TGF-α, and CD244 in the CHA and CSU groups. Groups compared with the Mann-Whitney test. *P* values are shown in the figure. N = 65 CHA/57 CSU. *CHA*, Chronic histaminergic angioedema; *CSU*, chronic spontaneous urticaria; *NPX*, normalized protein expression; *OSM*, oncostatin-M; *TGF-α*, transforming growth factor alpha; *TNF-α*, tumor necrosis factor alpha; *VEGF*, vascular endothelial growth factor.

TABLE IV. Autoimmune profile

	CHA (n = 67)*	CSU (n = 63)	*P* value
ANA (positive), N (%)	18 (26.87)	19 (30.16)	.7015
Anti-TPO (positive), N (%)	14 (20.90)	14 (22.22)	>.999
Anti-TG (positive), N (%)	8 (11.94)	8 (12.70)	>.999
BAT test† (positive), N (%)	1 (1.52)	19 (31.15)	**<.0001**
% of CD63+ basophils, mean (SD)	0.37 (0.98)	11.22 (20.55)	**<.0001**

ANA, Antinuclear antibodies; *BAT*, basophil activation test; *CHA*, chronic histaminergic angioedema; *CSU*, chronic spontaneous urticaria; *SD*, standard deviation; *TG*, thyroglobulin; *TPO*, thyroid peroxidase.
Bold values indicate statistical significance ($P < .05$).
*From 1 patient, there were not enough sera to perform the tests.
†Group sizes for the BAT test were: CHA, n = 67; CSU, n = 60.

their family history (10.29% vs 1.59% in CSU, $P = .0636$), whereas patients with CSU reported more cases of urticaria (1.47% in CHA vs 14.29% in CSU, $P = .0077$). CSU family history results include CSU with AE and CSU without AE although most of the cases belong to the first (6 CSU with AE and 3 CSU without AE). Both groups had similar family backgrounds of atopy or autoimmune diseases.

Clinical features

Regarding AE, no significant differences in duration of symptoms, duration of individual episodes, or relation to estrogen intake were observed (Table II). However, significant differences in the AE location were found. Lips and eyelids AE were significantly more frequent in CSU (95.2% lips, 74.6% eyelids) than in CHA (79.4% lips, $P = .0086$; and 51.5% eyelids, $P = .0071$). In contrast, tongue AE was significantly more frequent in CHA (58.8%) than in CSU (29%, $P = .0008$). Uvular AE was equally infrequent in both groups, and none of the patients had laryngeal edema nor needed intubation or tracheotomy. We did not find differences on NSAID symptom exacerbation, although there was a suggestive trend for being more common in CSU ($P = .0586$).

Blood count and biomarkers

Blood cell count and other blood biomarkers are summarized in Table III. We did not find significant differences in any CSU biomarker such as CRP, D-dimer, or ESR (Table III). IgE levels were significantly higher in CHA (166.7 kU/L) than in CSU (141.3 kU/L, $P = .0330$).

Regarding blood count, we found significant differences in neutrophil numbers (4.0 ± 1.5 in CHA vs 4.9 ± 1.8 in CSU, $P = .0051$) and in basophil numbers (0.045 ± 0.03 in CHA vs

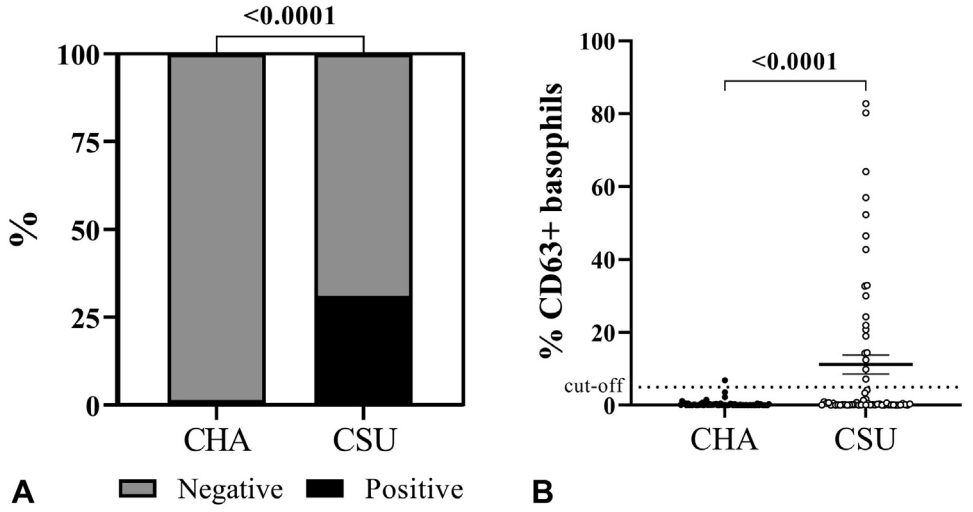

FIGURE 3. BAT test results for the CHA and CSU groups shown as (**A**) percentage of negative (gray) and positive (black) tests and (**B**) percentage of CD63+ basophils (CD123+ HLA-DR-), with the cutoff value shown with a dotted line (5%). Groups compared with the Fisher exact test and the Mann-Whitney test; *P* values are shown in the figure. *BAT*, Basophil activation test; *CHA*, chronic histaminergic angioedema; *CSU*, chronic spontaneous urticaria.

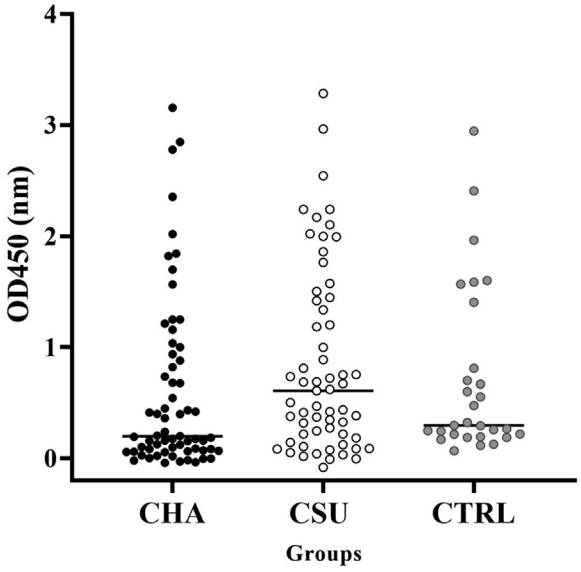

FIGURE 4. Example of an IgE anti-IL-24 ELISA for CHA (black dots), CSU (white dots), and controls (CTRL, gray dots). Results reported as OD values after blank subtraction and group mean. Groups compared with 1-way ANOVA with the Tukey post hoc test. ANOVA, Analysis of variance; CHA, chronic histaminergic angioedema; CSU, chronic spontaneous urticaria; OD, optical density.

0.032 ± 0.03 in CSU, $P = .0028$). Basopenia (described as $<0.01 \times 10^3$ basophils/mm^3) was more often observed in CSU than in CHA (CHA, N = 5 [7%]; CSU, N = 13 [20%]). When further analyzing these data, dividing CSU in those patients with an autoimmune mechanism assessed by the BAT, basopenia was more evident and almost unique to the autoimmune CSU group (Figure 1). This finding is interesting because basopenia and autoimmunity phenotype are related to severity[16] and more resistance to treatment.

Finally, we assessed 92 proteins in serum samples. Of those, 14 were below the detection limit in more than 50% of the samples and thus eliminated from further analysis. From the 78 analyzed proteins, 10 were differentially expressed between CSU and CHA: caspase-8 (CASP-8), CD244, CD8a, IL-6, oncostatin-M (OSM), osteoprotegerin (OPG), transforming growth factor alpha (TGF-α), tumor necrosis factor alpha (TNF-α), TNF-related apoptosis inducing ligand, and vascular endothelial growth factor (VEGF) (Figure 2). Of those, OPG, CASP-8, and CD244 were elevated in CHA when compared with CSU, whereas the rest were significantly higher in CSU.

Autoimmunity

Autoimmunity profiling features are described in Table IV. When assessing the ability of patients' sera to activate normal healthy basophils, we found that 31.15% of CSU sera induced basophil activation (Table IV and Figure 3). However, none of the CHA sera were able to activate normal basophils, except for 1 patient's serum (1.52%, $P \leq .0001$), which induced activation in 6.88% of the basophils, slightly over the 5% cutoff value. The mean of CD63 activation for CSU sera was 11.22%, whereas for CHA, it was 0.37% ($P \leq .0001$). Negative values for both groups ranged from 0% to 4.21% (with 95% of values between 0% and 2.8%). The conclusion is that CHA is not associated with IgG antibody to the IgE receptor or with IgG anti-IgE.

We did not find significant differences between the 2 groups regarding autoimmunity in other markers analyzed. Antithyroid antibodies were present in both CHA and CSU in the same proportion: 20.90% versus 22.22% for anti-peroxidase antibodies, 11.94% versus 12.70% for anti-thyroglobulin antibodies, in both cases, with normal thyroid function. However, 8 of 63 patients with CSU and 3 of 68 with CHA had thyroiditis. Concerning ANA, proportions were again very similar in both groups: 26.87% in CHA and 30.16% in CSU.

In addition, we addressed the presence of anti−IL-24 IgE antibodies with an ELISA (Figure 4). As no standard was available, serum samples from control individuals (no CSU or CHA

individuals) were used to evaluate reference values. Even though there was a trend for lower levels of IgE anti−IL-24 in CHA, no significant differences were observed between groups including the control group.

DISCUSSION

Recurrent AE without hives responding to antihistamines is classified as a nonhereditary type of AE,[1,2] but when dealing with urticaria, it is included in the definition of CSU[3]; in other words, the latter can include patients with wheals only, urticaria and AE together, or AE alone. CHA is easily distinguished from that associated with bradykinin production, such as hereditary AE or angiotensin-converting enzyme-inhibitor AE, and does resemble the AE we see associated with CSU. However, there is no explanation why some patients with histaminergic AE never have urticaria, and there is just as much rationale to consider them different entities as there is for differing subgroups of a single disorder. The observation that the AE is "histaminergic", that is, responsive to antihistamines used prophylactically, or more recently, responsive to omalizumab, does not mean that they have to have the same pathogenesis. Many different types of urticaria (mast cell dependent) respond to antihistamines and/or omalizumab, including dermographism and cold urticaria.[17-20] When the initiating stimulus for mast cell activation is clearly different, the various urticarias are considered different disorders. When initiating stimuli are less clear, one ought to rely on similar or differing pathogenesis to aid in classification. Finally, it has been reported that AE-predominant CSU refractory to high-dose antihistamines responds well to the addition of montelukast,[21] while this is not obvious for CSU patients with hives and AE.[3] These data also suggest the existence of differences in the pathophysiology of CHA versus CSU.

One important finding of this study is the sex distribution of the 2 disorders and the role of antibody to the IgE receptor as a pathogenic mechanism for the activation of mast cells. It is well known that CSU is more prevalent in women with a sex distribution of approximately 70:30 female/male.[22-24] It is true for those with urticaria alone as well as for the 40% to 50% with AE along with urticaria.[6] These 2 groups do not differ in the percent positivity of antibody to the IgE receptor. When we assessed patients with CHA, the sex distribution favored male sex, and this has been reported previously,[25-28] but largely ignored. Certainly, additional studies with larger numbers of patients in every category would help secure the information, but if these subpopulations represent the same disorder, the sex distributions should not differ.

Second, antibody to the IgE receptor is present at a rate of 25% to 45%[29-31] in CSU, depending on the study. A similar analysis of a pediatric population with CSU reported positivity of 47%, with 0% in children with atopic dermatitis included as a control group.[32] The percentage positivity reported herein is 31.5%, close to an average of reported values. The fact that essentially none of the patients with CHA have the antibody suggests that it plays no role and is our strongest evidence that they are different disorders. We also addressed other markers related to autoimmunity. The positive ANA are typically of a speckled pattern and are not associated with systemic lupus erythematosus in any of those subgroups. It has no pathogenic significance but suggests immune "activity." The same is true for IgG antithyroid antibodies,[33] which are elevated (approximately 25%) in all forms of CSU, and it is also true but to a lesser degree for CHA. IgE antithyroid antibodies are elevated in much higher numbers in CSU[34] compared to the incidence of IgG antithyroid antibodies but were not measured in this study. Such IgE antibodies might be pathogenic, based on the efficacy of omalizumab, but antigens other than the thyroid are more likely to be germane[35] as we do not have thyroid antigen in skin. Finally, we assessed a recently proposed CSU biomarker, anti−IL-24 IgE antibodies. In a previous study, these antibodies were more often observed in patients with CSU compared with controls.[35] We did observe a small percentage of patients with elevated levels of autoantibodies, more frequently in the CSU group compared with the CHA group, although differences were not significant. Importantly, a group of controls (including nonatopic and atopic donors) also had individuals with elevated levels of anti−IL-24 IgE. The lack of a standardized method precludes presenting absolute values; nevertheless, our observations suggest that it may not be a specificity biomarker for CSU so that the levels of IL-24 do not help resolve the issue we raise regarding the categorization of CHA.

Through targeted proteomics, we assessed 78 proteins with 10 of those presenting differences between CSU and CHA. Overall, patients with CSU seem to depict a higher inflammatory context, with proteins such as TNF-α or IL-6 elevated, or OSM, which is a regulator of IL-6. Other studies have shown before that IL-6 is increased in CSU when compared with control individuals,[36] and this cytokine has been suggested as a biomarker for disease activity. VEGF and TGF-α, 2 angiogenesis inducers, were also elevated in our CSU cohort versus CHA. Other studies have shown elevated VEGF levels in CSU compared with controls,[37] although there are conflicting data in the literature.[38] On the other hand, CD244 (also known as natural killer cell receptor 2B4) is a cell receptor expressed on lymphocytes, mast cells, and eosinophils. Elevated levels of soluble CD244 in CHA could be related to the tendency for higher levels of circulating eosinophils compared with CSU, which in turn could secrete higher levels of exosomes containing the receptor. The relevance of the observations regarding other protein differences seen for CSU versus CHA is unknown. We acknowledge that no *P* value correction has been applied despite performing multiple comparisons; however, we did that as we consider our study exploratory as it is the first study specifically focusing on CSU versus CHA. Further studies are needed to explore serum proteome of both groups and to verify our findings.

There are many parameters that do not differ between CSU and CHA. However, all are nonspecific. In addition to the autoimmunity-related parameters described above, the elevated sedimentation rate or CRP are not meaningful other than markers of cutaneous inflammation. The elevated D-dimer is also noninformative. For example, it is elevated in hereditary AE[39] where there is no role for mast cells or histamine and may reflect endothelial cell activation. Of course, none of these disorders have clinical thrombosis or evident fibrinolysis.

Lastly, we evaluated another important paradigm associated with CSU, namely, basopenia. It has been reported that 5% to 30% of patients have basopenia and hyporesponsive basophils to exogenous stimulation with anti-IgE[10,11,40-42] both of which reverse in response to therapy[43,44] or spontaneous remission.[42] The hyporesponsiveness is due to enhanced activity of phosphatases, which de-phosphorylate kinase enzymes needed for cell activation.[45,46] Thus, a molecular explanation is evident, and the

observation is either pathogenic or even if not, at least secondary to having CSU. In this study, only basopenia could be assessed, and the percentage of this abnormality in patients with CSU was approximately 20% (13 patients with $<0.01 \times 10^3$ basophils/mm^3), whereas the value for CHA was 7% (5 patients). One can argue that the cellular infiltrate associated with CSU and the diffuseness of symptoms is different from the localized nature of CHA and that the comparison may not be meaningful. However, if one considers all of our observations that pertain to pathogenesis: sex distribution, that is, estrogen effect, the incidence of antibody to the IgE receptor, and abnormalities of basophil counts, we see major differences when CSU with AE is compared with idiopathic histaminergic AE. By contrast, nonspecific abnormalities, for example, ESR, CRP, D-dimer, and ANA, that reflect inflammation or immune activity are similar, but do not reflect the pathogenesis of either disorder. In addition, when observing a family history of patients, it is noted that those with CHA had more antecedents of AE, whereas for patients with CSU, it was the opposite. These cases, although infrequent and being patient self-reported, suggest that genetic or epigenetic factors contributing to one disorder or the other are at least partially different.

Our data suggest that patient characteristics and underlying mechanisms involved in CSU and CHA differ, at least partially. The absence of an "autoimmune" subgroup (based on the BAT) demonstrates that, although antibodies against IgE or IgE receptor may contribute to the CSU pathophysiology, they are not essential for the appearance of AE. In addition, the more pronounced basopenia in CSU and the differences in some eosinophil-related proteins suggest differences in cell recruitment in the 2 disorders that should be explored.

We therefore conclude that CHA should not automatically be considered a subtype of the patient spectrum of CSU. This has been assumed to be the case for decades with no attempt to question whether the assumption is justified. We think it is not. What is needed are further studies that either support or refute what we propose. The assumption that they are the same disease pervades the literature and is not evidence-based.

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