

# Chronic Autoimmune Urticaria: Frequency and Association With Immunological Markers

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## ■ Abstract

**Background:** Chronic autoimmune urticaria (CAU), a subgroup of chronic idiopathic urticaria (CIU), is characterized by severe and persistent wheals accompanied by redness and itching. Diagnosis is almost completely based on clinical suspicion and the results of the autologous serum skin test (ASST).

**Objectives:** To determine the frequency of CAU and compare the clinical and laboratory parameters of patients with positive and negative ASST results.

**Patients and Methods:** A total of 165 patients with chronic urticaria (CU) were enrolled; 31 were excluded (known causes and pregnancy/breastfeeding), leaving 134 patients with CIU. A clinical evaluation and routine and specific laboratory tests were performed.

**Results:** The cause of CU was identified in 18.9% of patients; 81.2% patients were considered to have CIU. The ASST result was positive in 39.6% of patients with CIU, who had more frequent urticaria attacks than patients with a negative ASST result. Patients with positive results had a higher urticaria activity score than those with negative results, although the difference was not statistically significant. As for immunological markers, the absolute eosinophil count and serum immunoglobulin (Ig) E titer were lower in patients with a positive ASST result than in those with a negative ASST result, although, again, the difference was not statistically significant ( $P=.07$ ). Antithyroid antibody titer and B-cell percentage were higher in patients with a positive ASST result than in those with a negative result, and the difference was statistically significant ( $P=.04$  and  $.004$ , respectively).

**Conclusions:** ASST remains a baseline diagnostic test for CAU. Patients with CAU had more frequent attacks and higher antithyroid antibody titers and peripheral B-cell percentages, as well as lower absolute eosinophil counts and serum IgE concentrations.

**Key words:** Autologous serum skin test. Chronic urticaria. Autoimmunity.

## ■ Resumen

**Antecedentes:** La urticaria crónica autoinmunitaria (UCA), un subgrupo de urticaria crónica idiopática (UCI), se caracteriza por la presencia de habones de carácter grave y persistente acompañados de enrojecimiento y prurito. El diagnóstico se basa casi por completo en la sospecha clínica y los resultados de la prueba cutánea con suero autólogo (PCSA).

**Objetivos:** Determinar la frecuencia de la UCA y comparar los parámetros clínicos y de laboratorio de pacientes con resultados positivos y negativos en la PCSA.

**Pacientes y métodos:** Se incluyó a 165 pacientes con urticaria crónica (UC), de los que se excluyó a 31 (causas conocidas y embarazo/lactancia), quedando 134 pacientes con UCI. Se llevaron a cabo una evaluación clínica y análisis de rutina y específicos.

**Resultados:** La causa de la UC se identificó en un 18,9% de los pacientes; se consideró que un 81,2% de pacientes padecía UCI. El resultado de la PCSA fue positivo en un 39,6% de pacientes con UCI, que tenían episodios de urticaria con mayor frecuencia que los pacientes con un resultado negativo en la PCSA. Los pacientes con resultados positivos presentaron una puntuación de actividad de urticaria más elevada que aquellos con resultados negativos, si bien la diferencia no fue estadísticamente significativa. En cuanto a los marcadores inmunológicos, el recuento absoluto de eosinófilos y el título de inmunoglobulina (Ig) E en suero fueron más bajos en pacientes con un resultado positivo en la PCSA que en aquellos con un resultado negativo en dicha prueba, aunque, de nuevo, la diferencia no fue estadísticamente significativa ( $p=0,07$ ). El título de anticuerpos antitiroideos y el porcentaje de linfocitos B fueron mayores en los pacientes con un resultado positivo en la PCSA que en aquellos con un resultado negativo, y la diferencia fue estadísticamente significativa ( $p=0,04$  y  $0,004$ , respectivamente).

**Conclusiones:** La PCSA sigue siendo una prueba diagnóstica básica para la UCA. Los pacientes con UCA presentaron episodios más frecuentes, títulos de anticuerpos antitiroideos y porcentajes de linfocitos B periféricos más elevados, así como recuentos absolutos de eosinófilos y concentraciones séricas de IgE más bajos.

**Palabras clave:** Prueba cutánea con suero autólogo. Urticaria crónica. Autoinmunidad.

## Introduction

Chronic urticaria (CU) is a common skin disorder, affecting 0.1%-1% of the general population. It is characterized by recurrent and transitory (<24 hours) pruritic erythematous wheals that present at least twice weekly for at least 6 weeks [1]. Several investigators have demonstrated a subgroup of chronic idiopathic urticaria (CIU), chronic autoimmune urticaria (CAU), which affects 30%-50% of CIU patients [2-4].

Immune dysregulation in CAU is characterized by a systemic inflammatory profile associated with abnormal B-cell-induced immunoglobulin (Ig) E production and T-cell-induced cytokine production [5].

CAU is characterized by the presence of functional autoantibodies that activate mast cells and basophils. About 50% of these antibodies are functional IgG autoantibodies that bind to the high-affinity IgE receptor Fc $\epsilon$ RI, and 9% bind to the IgE antibody itself [6]. Their presence is consistent with disease exacerbation [7].

Diagnosis of CAU is based on clinical suspicion and in vivo and in vitro tests to determine functional histamine release from basophils or mast cells. The autologous serum skin test (ASST) is an in vivo test that assesses autoreactivity in CAU patients, particularly during the active phases of the disease. It may be an indication of mast cell-activating autoantibodies that act either indirectly through the release of mediators from cutaneous mast cells and other cells or directly in the microvasculature of the skin. The sensitivity of the test is approximately 70% and the specificity 80% [8]. Hence, the presence of functional autoantibodies should be confirmed by in vitro tests such as the basophil histamine release assay and immunoassay [9].

Despite being the gold standard for detecting functional autoantibodies, the basophil histamine release assay is a time-consuming procedure that is difficult to standardize [10]. Furthermore, attempts to develop successful Western blot assays and enzyme-linked immunosorbent assays continue to be unsuccessful [11,12].

Identification of an autoimmune cause can help to rule out the need to continue searching for other etiologies. Early diagnosis is important, as patients need high doses of antihistamines, systemic corticosteroids, and immunomodulatory drugs in severe cases [13]. Therefore, we aimed to determine the frequency of CAU among patients with CIU attending our Allergy and Immunology Unit and to identify any clinical and laboratory findings related to positivity of ASST.

## Patients and Methods

From December 2009 to October 2010, we recruited 165 patients with CU from the Allergy and Immunology Unit of the Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt. A diagnosis of CU was defined as recurrent episodes of wheals, with or without angioedema, of at least 6 weeks duration [14]. Patients had to be off antihistamines for at least 2-3 days (long-acting antihistamines for 7 days) [9,15]

and corticosteroids and other immunosuppressive drugs for 4 weeks before the study to reduce the possibility of generating false-negative results. The etiology of CU was evaluated using the medical history, clinical examination, and laboratory tests. The data recorded from the medical history included duration of disease, frequency of attacks, associated angioedema, dermographism, and history of atopy. Clinical examination included both general and dermatological examinations. Laboratory tests [16] included complete blood count, liver function tests, kidney function tests, and infection panel (hepatitis surface antigen, antibody titers for hepatitis B and C virus, throat culture, urine analysis and culture, and microscopic examination of stool for parasites). In order to rule out allergic causes of urticaria we performed skin prick tests with the following common allergens: house dust mite, human hair, tobacco smoke, wool, cotton, mixed fungus (*Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*), mixed pollens, and hay dust. Written informed consent was obtained from all patients.

### Exclusion Criteria

We excluded 31 patients who were pregnant or breastfeeding or had a known cause of CU.

The remaining 134 patients with CIU underwent the following evaluations:

1. Urticaria activity score (UAS) [17].
2. Peripheral blood eosinophil count. Eosinophilia was defined as a count >5% or >350 cells/mm<sup>3</sup> [18].
3. Serum total IgE level (Immundiagnostik IgE ELISA kit, Immundiagnostik AG, Bensheim, Germany).
4. Antithyroid peroxidase (anti-TPO) antibody (Medizym anti-TPO ELISA, Medipan Diagnostica, Dahlewitz/Berlin, Germany). All the procedures were performed according to the manufacturers' instructions. Anti-TPO antibody values of >30 IU/mL were considered positive.
5. Flow cytometry. The percentage of CD3<sup>-</sup>CD19<sup>+</sup> B lymphocytes in peripheral blood was detected. Two-color analysis was performed using monoclonal antibodies marked with CD19-fluorescein isothiocyanate (HIB19 clone; BD Pharmingen, San Diego, California, USA) and CD3-allophycocyanin (UCHT1clone; BD PharMingen) according to the manufacturer's instructions. Cells were analyzed using a FACScan flow cytometer (Becton Dickinson, San Diego, California, USA) and CellQuest software (Becton Dickinson, Mountainview, California, USA).
6. ASST technique. Patients received an intradermal injection (50 µL of autologous serum, histamine diphosphate, and sterile physiological saline) into the volar forearm [15,19], avoiding areas known to have had spontaneous wheals in the previous 48 hours (mast cells may be refractory to further activation [local tachyphylaxis]) [20]. After 30 minutes (15 minutes for histamine), the wheal was measured at its 2 longest perpendicular diameters and the average was calculated [6]. A positive ASST result was defined as a serum-induced wheal with a diameter of ≥1.5 mm as compared to a saline-induced wheal at 30 minutes (Figure).



**Figure.** A positive result in the autologous serum skin test. Serum was injected more proximally and histamine more distally, with normal saline in the middle. A significant wheal and flare response was seen at the serum and histamine injection sites only. The diameter of the serum-induced wheal is 1.7 mm greater than that of the saline-induced wheal.

**Table 1.** Characteristics of Chronic Idiopathic Urticaria Patients

	Positive ASST (n=53)	Negative ASST (n=81)	P <sup>a</sup>
Age, y			
Range			
Mean (SD)	18-65 36.4 (10.8)	20-75 39.7 (14.3)	.15
Sex			
Females, No. (%)	38 (71.7%)	46 (56.8%)	.08
Males, No. (%)	15 (28.3%)	35 (43.2%)	
Duration of the disease, mo			
Range	3-121	1-300	.09
Mean (SD)	27.4 (31)	26.7 (44.3)	
Frequency of attacks, >5/wk, No. (%)	49 (92.5%)	52 (52.8%)	<.001
Angioedema, No. (%)	6 (11.3%)	8 (9.9%)	.78

Abbreviation: ASST, autologous serum skin test.

<sup>a</sup>P≤.05 was significant

**Table 2.** Urticaria Activity Score

Parameter	Positive ASST (n=53)	Negative ASST (n=81)	P <sup>a</sup>
Number of wheals	2.5 (0.6)	2.3 (0.7)	.08
Size of wheals	2.9 (1.0)	2.6 (0.9)	.07
Intensity of pruritus	2.8 (0.8)	2.7 (0.9)	.05
Duration	2.3 (0.9)	2.0 (0.8)	.18
Frequency	2.7 (1.0)	2.5 (1.1)	.28
Frequency of antihistamine use	2.4 (0.9)	2.3 (0.7)	.47
TSS	15.2 (5.1)	13.8 (4.1)	.07

Abbreviations: ASST, autologous serum skin test; TSS, total symptom score.

<sup>a</sup>P≤.05

### Statistical Analysis

Data were recorded and processed using SPSS version 12.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative variables were expressed as mean (SD) and compared using the Mann-Whitney test for 2 independent variables. Qualitative variables were expressed as frequency and percentage and compared using the  $\chi^2$  test or Fisher exact test when appropriate. A P value <.05 was considered significant.

### Results

A total of 165 patients with CU were enrolled in this study. The etiology—or an exclusion criterion—was identified in 31 (18.9%) patients, and the remaining 134 (81.2%) patients were considered to have CIU. The ASST result was positive in 53 (39.6%) CIU patients and negative in 81 (60.4%) (Table 1). There

were no significant differences between the groups in age, sex, total duration of disease at the time of assessment, and angioedema. The number of frequent attacks (>5 times/week) was significantly greater in the positive group than in the negative group ( $P<.001$ ). The UAS was higher in patients with a positive ASST result (Table 2), although the difference was not significant ( $P=.07$ ). Immunological markers are shown in Table 3. The absolute eosinophil count and serum IgE titer were lower in patients with a positive ASST score than in those with a negative score, although the difference was not significant ( $P=.22$  and .15, respectively).

**Table 3.** Immunological Markers

	Positive ASST (n=53)	Negative ASST (n=81)	P <sup>a</sup>
Eosinophil count/mm <sup>3</sup>			
Range	60-211	76-269	
Mean (SD)	146 (65.8)	160.6 (69.2)	.22
Serum IgE level, IU/mL			
Range	42.13-110.25	54.23-127.65	
Mean (SD)	67.4 (44.22)	82.6 (40.07)	.15
Anti-TPO antibody			
No. of patients, %	8 (15.1%)	4 (4.9%)	.04
B cells, % <sup>b</sup>	16.38 (5.97)	12.22 (9.25)	.004

Abbreviations: ASST, autologous serum skin test; Ig, immunoglobulin; TPO, thyroid peroxidase.

<sup>a</sup>P≤.05.

<sup>b</sup>B cells/peripheral blood lymphocytes.

Higher anti-TPO antibody titers and B-cell percentages were recorded in patients with a positive ASST result than in those with a negative result, and the difference was significant ( $P=.04$  and  $.004$ , respectively).

## Discussion

The ASST showed that CAU affected 39.6% of our patients. The prevalence of positive ASST results in CU patients has been reported to vary from 34% to 67% in various studies [21-23]. Differences in frequency according to ethnic group suggest a genetic background [9]. In addition, ASST may generate false-positive results owing to the presence of mast cell—specific nonimmunoglobulin histamine—releasing factors [24] and clotted blood generates bradykinin [6]. We found no significant differences between the groups for age or associated angioedema. This finding agreed with the previous study of Kulthanian et al [25]. However, our study did detect a female predominance in CAU patients, a finding that is consistent with those of Caproni et al [21]. We found no significant differences in disease duration between the 2 groups, as was the case with George et al [26] in India. By contrast, Boguniewicz [27] found that disease duration was significantly longer in patients with a positive ASST result than in patients with a negative result. Frequent attacks (>5 times/week) were significantly more common in ASST-positive patients than in ASST-negative patients. This finding was consistent with the results of George et al and could be explained by the difficulty in controlling CAU.

Our study showed that the severity of urticaria was greater, although not significantly so, if the ASST result was positive. This is consistent with the findings of Bajaj et al [17]. However, Caproni et al [21] found that patients with a positive ASST result presented more severe clinical features than those with a negative result.

As for immunological markers, absolute eosinophil count was not significantly lower in patients with a positive ASST result, although it was within the upper limits of normal. This reduction is consistent with the accumulation of eosinophils in lesional skin [28]: eosinophils are recruited following the release of cytokines and chemotactic factors and activation and recruitment of adhesion molecules on migrating eosinophils and on endothelial cells [29]. The role of tissue eosinophilia is unclear, although it is possible that release of toxic major basic protein and eosinophil cationic protein further augments histamine release from mast cells in the late phase of the urticarial wheal [9].

We found that a positive ASST result was more likely to be associated with significantly lower IgE levels than ASST-negative patients. This finding agrees with those of Huilan et al [5], who attributed this association to IgE-anti-IgE immune complex formation that reduces the amount of detectable free IgE in patients with anti-IgE autoantibodies. In contrast, other authors showed serum IgE level to be significantly higher in ASST-positive patients [6,30], and this could be due to an improvement in CAU patients after treatment with the anti-IgE antibody omalizumab, which selectively binds to IgE, thus decreasing IgE receptor density on basophils and cutaneous mast cells and preventing activation by autoantibodies [6].

The significant association between a positive ASST result and anti-TPO antibody titer is consistent with the findings of other studies and can be explained by segregation of anti-TPO antibodies from IgE receptor antibodies because of B-cell hyperreactivity [23,31]. However, other authors did not detect a difference in the incidence of thyroid disease, probably as a result of insufficient sample size (thyroid autoimmunity occurs in less than 6% of the general population) [32]. Finally, we found that ASST-positive CAU patients were more likely to be associated with a higher percentage of B cells. This finding agrees with those of Huilan et al [5] and Toubi et al [33], who reported increased proliferation rates and decreased apoptosis rates for B cells.

In conclusion, ASST is a baseline diagnostic test for CAU. Patients with CAU have more frequent attacks and higher anti-TPO antibody titers and peripheral B-cell percentages, as well as lower absolute blood eosinophil counts and serum IgE titers.

Thyroid function and anti-TPO antibody titers should be routinely assessed in CU patients. In addition, successful therapy for urticaria should target the regulatory pathway linking B cells and IgE in order to downregulate Fc $\epsilon$ RI expression.

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