# CLINICAL FEATURES AND RELATED FACTORS IN PATIENTS WITH CHRONIC SPONTANEOUS URTICARIA WITH NEGATIVE AUTOLOGOUS SERUM TEST

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## ABSTRACT

**Objectives:** Chronic spontaneous urticaria (CSU) is a subtype of urticaria characterized by sustained mast cell activation. Approximately 50% of chronic spontaneous urticaria cases are thought to be associated with an autoimmune mechanism. The autologous serum skin test (ASST) is an in vivo laboratory assessment of mast cell degranulation. ASST can be used for screening patients with autoimmune chronic urticaria. This study aimed to investigate the clinical and laboratory characteristics, as well as associated factors, in CSU patients with a negative ASST result.

**Materials and methods:** A retrospective cross-sectional study was conducted on 711 medical records of patients who met all the diagnostic criteria for chronic spontaneous urticaria and underwent autologous serum testing. The medical records provided complete information on the patients' medical history, clinical manifestations, test results, and treatment responses.

**Results:** Among 711 patients with CSU, 55.7% (396/711) had a positive ASST result, while the remaining 44.3% (315/711) were negative. Among patients with a positive ASST result, females accounted for 63.38%, while males accounted for 36.62%. The prevalence of angioedema in the ASST-negative group was 23.5% which was lower than in the ASST-positive group (32.75%) ($p < 0.05$). There were no statistically significant differences in age, disease duration, serum IgE concentrations, or treatment response between the ASST-negative and ASST-positive groups.

**Conclusions:** The ASST is a simple test that may help support the diagnosis and management of chronic spontaneous urticaria.

**Keywords:** *ASST, autologous serum skin test, chronic autoimmune urticaria, chronic idiopathic urticaria, urticaria.*

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## 1. INTRODUCTION

Urticaria is a common skin disorder that affects up to 20% of individuals at some point during their lifetime.[1] The disease is characterized by wheal and/or angioedema accompanied by pruritus and typically resolves within 24 hours.[2,3] Urticaria is classified into acute urticaria and chronic urticaria based on disease duration: Acute urticaria is defined as urticaria lasting less than 6 weeks, whereas chronic urticaria is defined as urticaria recurring at least 2 days per week for a minimum of 6 weeks. Chronic urticaria is further subdivided into chronic spontaneous urticaria (CSU) and chronic inducible urticaria (CindU).[4]

The pathogenesis of chronic urticaria is complex and may be triggered by drugs, physical stimuli, which occur as part of inflammatory or genetic disorders, or be of idiopathic origin.[5] Autoimmune mechanisms are suggested to underlie approximately half of chronic urticaria cases, characterized by the presence of autoantibodies that activate mast cells and basophils. Well-recognized autoantibodies include immunoglobulin G (IgG) autoantibodies directed against the α-subunit of the high-affinity IgE receptor (FcεRI) and anti-IgE antibodies. The basophil histamine release assay (BHRA) is considered the "gold standard" for detecting functional antibodies in the serum of patients with chronic urticaria; however, its application in clinical practice remains limited in many countries worldwide.

The autologous serum skin test (ASST) is a simple in vivo diagnostic procedure used to detect histamine release activity from mast cells. ASST is considered one of the adjunctive tests in the diagnosis of type IIb autoimmune chronic spontaneous urticaria (type IIb aiCSU). Several reports have demonstrated that patients with a positive ASST response tend to have higher disease activity, longer disease duration, greater impairment in quality of life, and an increased likelihood of concomitant angioedema.[6] Previous studies have suggested a possible association between ASST positivity and certain clinical characteristics of CSU.[6] This study aimed to analyze the clinical characteristics and associated factors in patients with chronic spontaneous urticaria who had negative ASST results.

## 2. MATERIALS AND METHODS

### 2.1. Study subjects

We reviewed medical records of patients diagnosed with chronic spontaneous urticaria (CSU) who were examined and treated at the Urticaria Clinic and the Inpatient Department of the National Hospital of Dermatology and Venereology between May 2023 and July 2024.

Inclusion criteria: Medical records of patients diagnosed with chronic spontaneous urticaria who underwent the autologous serum skin test, with complete clinical, paraclinical, and follow-up information. Exclusion criteria: Medical records were excluded for concomitant inducible urticaria, missing data, or inadequate laboratory results.

### 2.2. Research methods

***Study design***

A retrospective, cross-sectional study was conducted from May 2023 to July 2024 at the National Hospital of Dermatology and Venereology, including all medical records of chronic spontaneous urticaria patients who underwent the autologous serum skin test with complete information.

***Procedures***

*Step 1*: Selection of medical records was based on inclusion criteria and exclusion criteria.

*Step 2*: Collect data from medical records, including:

- Demographic characteristics: Age, sex, occupation.
- Clinical features: Onset time, disease severity (very mild, mild, moderate, severe), and accompanying manifestations.
- Related factors: Medical history (allergic, autoimmune, etc.), family history.
- Laboratory tests: Complete blood count (eosinophils, basophils), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), total IgE, serum T3, T4, TSH, and anti-TPO antibodies.
- Disease control-related factors: Antihistamine dose, control status (complete control, good control, poor control).

*Step 3*: Data entry and statistical analysis.

*Step 4*: Report results.

**Evaluation of treatment outcomes**

Baseline Disease Activity Score (UAS7): The Urticaria Activity Score over 7 days (UAS7) ranges from 0 to 42 and is categorized into levels of disease activity as follows:

- No wheals or pruritus: UAS7 = 0.
- Very low disease activity, well-controlled: UAS7 = 1 - 6.
- Low disease activity: UAS7 = 7 - 15.
- Moderate disease activity: UAS7 = 16 - 27.
- High disease activity: UAS7 = 28 - 42.

Disease Control Assessment Index (UCT): The Urticaria Control Test (UCT) is used to evaluate disease control at baseline (UCT T0) and after two weeks of treatment (UCT T2). The UCT score ranges from 0 to 16 and is interpreted as follows:

- Poor disease control: UCT < 12.
- Good disease control: UCT = 12 - 15.
- Complete disease control: UCT = 16.

Treatment Response Following Antihistamine Administration: Treatment response is classified into three categories based on changes in UAS7 and UCT scores:

- Complete response: The patient exhibits no wheals or pruritus, with UAS7 = 0 and UCT between 12 and 16.
- Partial response: The patient continues to experience wheals and/or pruritus, but symptoms have improved compared to baseline; UAS7 < UAS7 T0 and UCT < 12.
- No response: There is no improvement in wheals or pruritus; UAS7 ≥ UAS7 T0 and UCT < 12.

**Data analysis**

Data were encoded and analyzed using SPSS version 20.0. Quantitative data were presented as mean ± standard deviation (for normally distributed data), median and skewness, median, minimum, maximum, percentage, and frequency. Comparisons between two means were performed using the t-test for normally distributed variables and non-parametric tests (Wilcoxon signed-rank test or Mann-Whitney U test) for variables with non-normal distributions. A p-value < 0.05 was considered statistically significant Ethical Considerations

This study received the NHDV Institutional Review Board approval and adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational studies.

## 3. RESULTS

### 3.1. Patient age characteristics

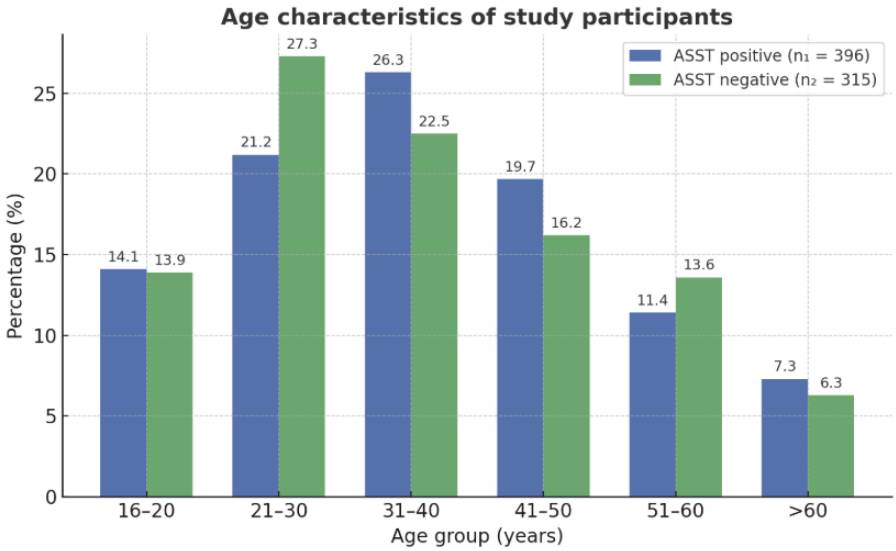


***Figure 1. Patient distribution by age (N = 711)***

Among the 711 patients, the predominant age groups were 21 - 30 years and 31 - 40 years. The average age of ASST-positive group is 37.2 ± 14.1 years, while that of the ASST-negative group was 36.3 ± 14.4 years (p = 0.429).

### 3.2. Patient distribution by sex

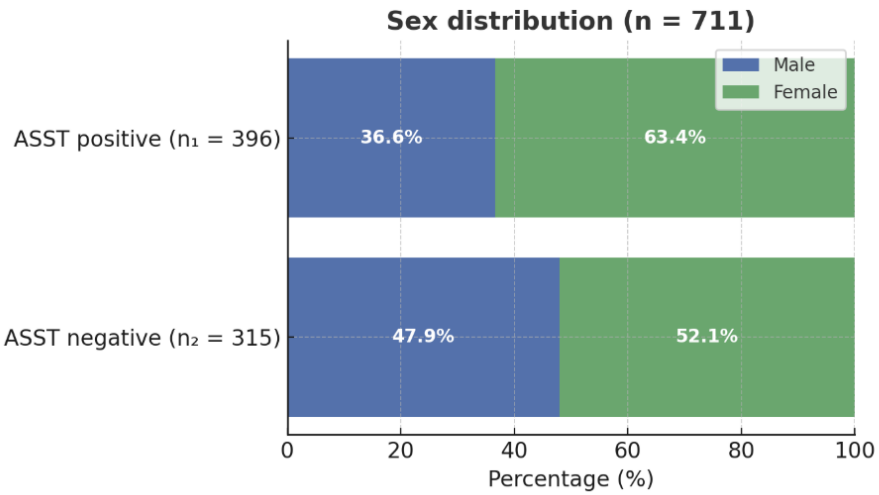


***Figure 2. Patient distribution by sex (N = 711)***

Among patients with a positive ASST result, 63.38% were female and 36.62% were male. whereas the ASST-negative group showed an almost equal sex distribution. This difference was statistically significant (p = 0.02).

### 3.3. Distribution characteristics of ASST results

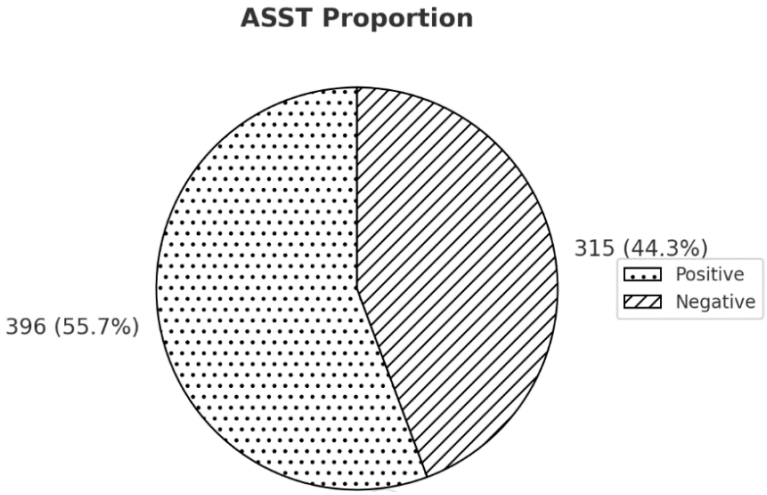


***Figure 3. Distribution of ASST rates in patients with CSU (N = 711)***

In our study, a total of 711 patients diagnosed with chronic spontaneous urticaria underwent the ASST. Of these, 396 patients (55.7%) had a positive ASST result, whereas 315 patients (44.3%) had a negative result.

### 3.4 Characteristics of disease duration

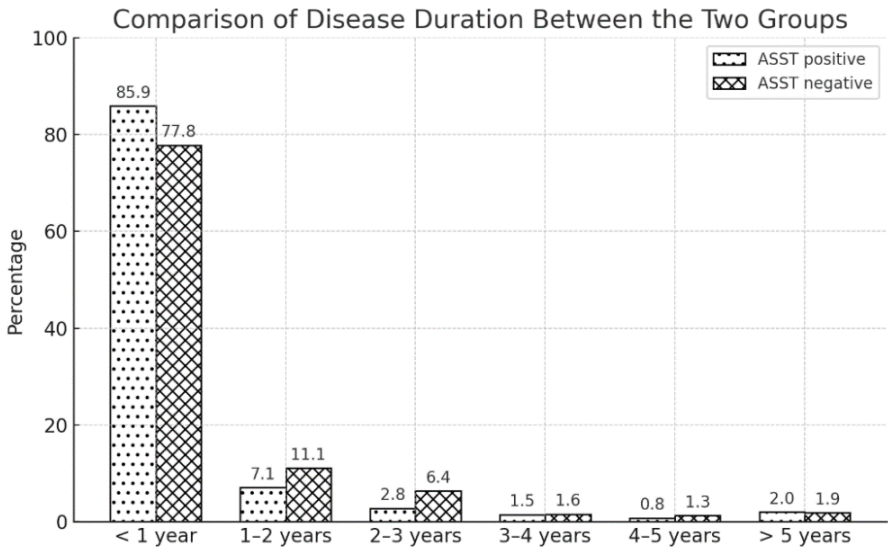


***Figure 4. Comparison of disease duration between the two groups (N = 711)***

The average disease duration in both groups was predominantly less than one year, with no difference in mean disease duration between the ASST-negative and ASST-positive groups.

### 3.5. Clinical characteristics of angioedema

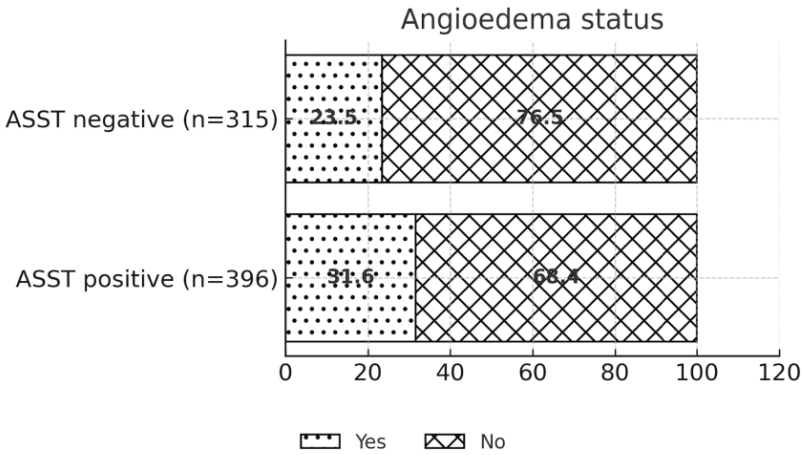


***Figure 5. Concomitant angioedema status (N = 711)***

In the ASST-positive group, the proportion of patients with angioedema was higher at 31.57% compared to 23.49% in the ASST-negative group; this difference was statistically significant (p = 0.017).

### 3.6. Characteristic changes in laboratory parameters

***Table 1. Changes in selected laboratory parameters (N = 711)***

| Parameter | ASST positive (n1 = 396) | | ASST negative (n2 = 315) | | p* |
|---|---|---|---|---|---|
| | n | % | n | % | |
| Eosinopenia | 76 | 19.19 | 46 | 14.6 | 0.107 |
| Basopenia | 43 | 10.86 | 21 | 6.67 | 0.052 |
| Abnormal FT3 | 19 | 4.8 | 21 | 6.66 | 0.52 |
| Abnormal FT4 | 15 | 3.79 | 20 | 6.35 | 0.117 |
| Abnormal TSH | 23 | 5.81 | 10 | 3.17 | 0.097 |
| Abnormal Anti-TPO | 85 | 21.46 | 77 | 24.44 | 0.347 |
| IgE elevated | 263 | 66.41 | 221 | 70.16 | 0.287 |
| IgE normal | 133 | 33.59 | 94 | 29.84 | |

**Chi-square test.*

No statistically significant differences were observed in eosinophil counts, basophil counts, thyroid function test results, or serum IgE levels between ASST-negative and ASST-positive groups (p > 0.05).

### 3.7. Characteristics of treatment response

***Table 2. Comparison of treatment response between the two groups after 2 weeks***

| Evaluation criteria | | ASST-positive n1 = 396 | | ASST-negative n2 = 315 | | *p** |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Antihistamine dose | Standard dose | 228 | 57.57 | 169 | 53.65 | 0.056 |
| | Increased dose | 168 | 42.43 | 146 | 46.35 | |
| Response level | Complete response | 167 | 42.17 | 137 | 43.49 | 0.058 |
| | Partial response | 84 | 21.21 | 60 | 19.05 | |
| | No response | 145 | 36.62 | 118 | 37.46 | |
| Control level | Good control | 237 | 59.85 | 164 | 52.06 | 0.068 |
| | Poor control | 159 | 40.15 | 151 | 47.94 | |

**Chi-square test.*

There was no significant difference in the level of treatment response and disease control between ASST-negative and ASST-positive patient groups ($p > 0.05$).

## 4. DISCUSSION

ASST is a simple *in vivo* method used for the diagnosis of type IIb aiCSU. It is performed by obtaining a serum sample from patients with chronic urticaria and subsequently injecting it intradermally into uninvolved skin. A positive result is evidenced by the appearance of an erythematous wheal within 30 minutes.

ASST plays an important screening tool and is commonly used alongside the basophil histamine release assay (BHRA) in the diagnosis of type IIb aiCSU. The negative predictive value of ASST has been reported as 82.5 ± 14%, indicating that patients with chronic urticaria who test negative are unlikely to have circulating autoantibodies.[7] However, a positive ASST result may also occur in other allergic conditions and even in healthy individuals. Therefore, confirmation of autoimmune involvement requires more specific and high-value quantitative assays. In our study, 711 patients with CSU underwent the ASST, of whom 55.7% tested positive and 44.3% tested negative. Among the assessed patients, there were 296 males and 415 females. The mean age in the ASST-positive group was 37.19 ± 14.06 years, compared to 36.29 ± 14.42 years in the ASST-negative group. Among patients with a positive ASST result, 63.38% were female and 36.62% were male. whereas the ASST-negative group showed an almost equal sex distribution ($p < 0.05$). CSU is characterized by mast cell and/or basophil activation, triggering an inflammatory response. Reproductive hormones modulate immune cell function and inflammation, including mast cell activity. Consequently, CSU may be associated with hormonal changes, including endocrine disorders, menstrual cycle phases,

pregnancy, menopause, and the use of oral contraceptives or hormone replacement therapy.[5] Dehydroepiandrosterone (DHEA) is recognized as a key modulator of both endocrine and immune functions, and a decline in DHEA levels may exert multiple physiological consequences. In patients with chronic spontaneous urticaria (CSU), serum concentrations of DHEA sulfate are significantly reduced compared with those of healthy controls and show a positive correlation with autologous serum skin test (ASST) reactivity. This observation aligns with current evidence indicating a predominance of female patients among those with ASST-positive CSU. Prior studies have reported that approximately 40% - 50% of CSU cases were associated with autoimmune mechanisms, with a higher prevalence in females.[2] Consistently, Aktar et al. (2015) reported that females accounted for 78% of the ASST-positive group, whereas males comprised only 22%.[8]

Angioedema, a clinical manifestation of urticaria, is characterized by an abrupt, well-circumscribed swelling of the subcutaneous and/or submucosal tissues. In the context of urticaria, it is caused by non-specific histamine release from activated mast cells. Although not a direct marker of disease severity, angioedema has been associated with prolonged disease duration. Earlier research has shown that patients with a positive ASST exhibited higher urticaria activity scores (UAS7), longer disease duration, and an elevated risk of concurrent angioedema.[9,10] Our results observed that patients with a negative ASST had a lower prevalence of concomitant angioedema compared to those with a positive ASST (23.49% vs. 31.52%), with the difference reaching statistical significance ($p < 0.05$). These findings are consistent with those reported by Nui et al., who evaluated 2,554 patients undergoing ASST in the Asian population. However, in our current study, no statistically significant differences were found between the two groups regarding age at disease onset, disease duration, or disease activity as assessed by the UAS7 score, consistent with findings from previous studies.[6,7,11]

Immunoglobulin E (IgE) plays a pivotal role in the pathogenesis of CSU, with autoantibodies directed against either the high-affinity IgE receptor (FcεRI) or IgE itself. Mast cell activation in chronic urticaria can occur via both IgE-dependent (e.g., allergens, anti-IgE) and IgE-independent pathways. Autoantibodies to IgE and the α-chain of FcεRI are implicated in disease development. Approximately 30% of patients with chronic urticaria present with elevated total IgE levels, and higher serum IgE concentrations have been associated with greater disease severity and prolonged disease duration.[12,13] Our cohort's elevated serum IgE levels were seen in 66.41% of patients in the ASST-positive group and 70.16% of those in the ASST-negative group. However, the difference between the two groups was not statistically significant. The association between ASST reactivity and the presence of functional autoantibodies targeting IgE or its high-affinity receptor (FcεRI) has been documented in a study conducted approximately two decades ago, which included more than 300 patients diagnosed with CSU.[14,15] In this investigation, ASST yielded positive results in 67% of patients, whereas the basophil histamine release assay (BHRA) was positive in only 16.5%. Notably, all BHRA-positive patients also demonstrated a positive ASST, whereas only 22% of ASST-positive individuals were BHRA-positive. These studies indicate that all BHRA-positive patients also exhibit a positive ASST result. The BHRA is currently considered the gold standard for detecting functional autoantibodies in the serum of patients with chronic urticaria. However, its procedure is challenging to standardize, as it requires fresh basophils from healthy donors, is time-consuming, and remains

limited to specialized research centers. Therefore, the ASST is regarded as a simple test that can aid in the diagnosis of autoimmune urticaria.

Thyroid disease is also among the most frequently reported comorbidities in patients with CSU. In our findings, the prevalence of anti-thyroid peroxidase antibodies (Anti-TPO) was 21.46% in the ASST-positive group and 24.44% in the ASST-negative group, with no statistically significant difference between the two groups. Patients with both thyroid dysfunction and CSU experienced more severe and prolonged episodes of urticaria compared to those without thyroid dysfunction. A recent study reported hypothyroidism in 9.8% of CU patients, compared to 0.6% in the control group.[16,17] In a study of 110 CSU patients, Kumar et al. (2016) found no statistically significant differences between ASST-positive and ASST-negative groups in the frequency of urticaria episodes or the prevalence of thyroid disorders.[18]

The ASST has a negative predictive value of 80-90%, with a negative result serving as a strong prognostic indicator for achieving urticaria remission within two years. In our study, the ASST-positive and ASST-negative groups had comparable outcomes: Response rates to standard-dose antihistamines were 57.57% and 53.65%, complete response rates were 42.17% and 43.49%, and good disease control was achieved in 59.85% and 52.06% of patients, respectively.[18] None of these differences reached statistical significance. The study by Kumar et al. (2016) on 110 patients with CSU (48 ASST-positive and 62 ASST-negative) also showed that, in the ASST-positive group, 31 patients (81.25%) improved with standard-dose antihistamines compared to 30 patients (43.54%) in the ASST-negative group; however, the difference was not statistically significant. The study by Ana Maria (2024) identified several factors that may predict treatment response in patients with CSU. Factors such as high disease activity, low serum IgE levels, elevated CRP or ESR, ASST positivity, BAT/BHRA positivity, reduced basophil count, reduced eosinophil count, and elevated D-dimer were associated with poor response to antihistamines but better response to omalizumab and cyclosporine. In addition, baseline IgE and FcεRI expression within the normal range or slightly elevated were predictive of a faster response to omalizumab. However, to date, no predictive factors have been fully validated, and none are currently recommended for routine use.

In summary, current data indicate that CSU patients with a positive ASST are more likely to present with concomitant angioedema and have a higher likelihood of coexisting autoimmune conditions compared to those with a negative ASST.

## 5. CONCLUSIONS

ASST is considered a screening test for the diagnosis of type IIb aiCSU and can assist in classifying the clinical subtypes of CSU. Among patients with CSU, those with a negative ASST have a lower risk of angioedema and a lower prevalence of type IIb aiCSU.

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Conflict of interest statement: The authors declare no conflicts of interest related to this work.

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