

12-30-2023

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### Recommended Citation

Shantharaju, Sanjana Agrahara; Hongal, Amrita; Gupta, Diksha; and Jonnagadla, Girishma (2023)  
"Favorable benefits of autologous serum therapy in chronic spontaneous urticaria," *Journal of General -  
Procedural Dermatology & Venereology Indonesia*: Vol. 7: Iss. 2, Article 3.  
DOI: 10.7454/jdvi.v7i2.1149  
Available at: <https://scholarhub.ui.ac.id/jdvi/vol7/iss2/3>

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### Cover Page Footnote

None

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## Favorable benefits of autologous serum therapy in chronic spontaneous urticaria

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### Abstract

**Background:** In chronic spontaneous urticaria (CSU), autologous serum therapy (AST) is an alternative treatment modality that provides promising benefit of extended relief period without side effects and economic burden of pills. This study aimed to assess the efficacy of AST in CSU patients irrespective of their autologous serum skin test (ASST) result.

**Methods:** Patients were enrolled based on inclusion and exclusion criteria after taking written informed consent. A detailed history and clinical examination & relevant investigations were done. ASST was performed and irrespective of the test result AST was given to all study subjects that included weekly intramuscular injections for 8 times. Urticaria activity score (UAS), urticaria severity score (USS) and dermatology life quality index (DLQI) were recorded at baseline and 8th visit to note the improvement in the condition.

**Results:** Among 34 patients, 24 patients (70.58%) had a positive ASST result while 10 (29.41%) were negative. In 24 patients with positive ASST result, the average UAS, USS, & DLQI was 2.95, 20.45, & 6.6 respectively at baseline and improved to 0.29, 1.83, & 0.45 respectively at week 8. In 10 patients with negative ASST, the average UAS, USS, and DLQI was 2.4, 20.9, & 8 respectively at baseline and improved to 0.4, 2.8, & 0.6 respectively at week 8 after AST. Irrespective of ASST result, improvement was observed in UAS, USS, & DLQI, which is statistically significant.

**Conclusion:** AST is a beneficial alternative therapy in CSU patients with partial control of symptoms with pills.

**Keywords:** *autologous serum skin test, autologous serum therapy, chronic spontaneous urticaria*

### Background

The use of autologous serum is increasing, especially for diseases such as chronic spontaneous urticaria, atopic dermatitis, and vascular diseases. Autologous serum tears are also used to treat ocular surface disorders. Autologous whole blood or serum injections have long been used in the treatment of chronic urticaria. With a prevalence of 1.1% of the global population, urticaria is a relatively common skin disease; it can be of autoimmune etiology or idiopathic.<sup>1</sup> The cause of acute urticaria (lasting <6 weeks) can be easily-identified, while an exhaustive work up is needed for chronic urticaria (CU) (lasting >6 weeks). CU is a skin disease where wheals/hives, angioedema, or both appear almost every day that last for more than 6 weeks, resulting from mast

cells degranulation. Chronic inducible urticaria (CIU) and chronic spontaneous urticaria (CSU) are the broad sub-types of chronic urticaria.<sup>2</sup>

Two autoimmune mechanisms have been described in literatures for the pathogenesis of CSU: Type I autoimmune (auto-allergic) CSU is associated with immunoglobulin E (IgE) antibodies against autoantigens like thyroid peroxidase and IL-24, and type IIb autoimmune CSU is mediated by autoantibodies activating mast cells via IgE and FcεRI receptors.<sup>3</sup> This condition presents a challenge for the physician to investigate and evaluate the cause of chronic urticaria and for the patient because of poor quality of life (QoL) due to the irritable itch and high antihistamine burden.<sup>4</sup> Psychiatric comorbidities are common in patients with chronic urticaria, and the relationship between

the two entities is not fully understood. Antihistamines are the main treatment for both acute and chronic urticaria. The benefits of antihistamines are only limited to the period of its use, hence their safety for long-term use is questionable. In addition to compliance, there will be an economic burden of antihistamine pills as the disease course is relentless and unpredictable. The second line of drugs are immunosuppressants like corticosteroids, methotrexate, azathioprine, cyclosporine, which need regular follow-ups and laboratory monitoring.

The specific treatment options are omalizumab and other anti-IgE monoclonal antibodies, which can provide extended relief in patients in whom IgE is classically elevated. The efficacy of omalizumab has been proven in several studies.<sup>5</sup> Considering the cost of biological agents, there is a need for an alternative treatment modality that provides a longer remission period, lesser side-effect profile, and is more economical. Autologous serum therapy (AST) is an option with promising benefit of extended relief period. ASTs work by generating tolerance towards developing anti-idiotypic antibodies to antigens on mast cell that result in their degranulation.<sup>6</sup> Autologous serum skin test (ASST) is an intradermal test performed to evaluate autoreactivity where a wheal-and-flare reaction towards the intradermal autologous serum injection is observed. Autoreactivity, including auto-immunity and autoallergy, is specifically mediated by IgE autoantibodies to self-antigens.<sup>7</sup> ASST is a simple and practical test to detect autoreactive antibodies/ factors. However, recruiting patients for AST based on ASST can be misleading. A false positive ASST is usually observed due to the release of bradykinin and C5a activation; thus, ASST is not a valid indicator for the initiation of AST. This study aims to investigate the utility of AST in CSU patients, irrespective of their ASST results.

## Methods

This study was conducted after obtaining approval from the Institutional Ethics Committee (ethical clearance Number: BSGGIMS/IEC/App/Feb/ 2022/ 14). The cross-sectional study was conducted from November 2021 to November 2022.

Patients aged >18 years of both genders who have been suffering from CSU (3 or more episodes of wheals and itching occurring almost daily for >6 months) visiting the dermatology outpatient clinic block at our tertiary care teaching hospital were included in this study. Exclusion criteria include CIU, pregnancy and lactation, patients on drugs or

with diseases causing immunosuppression, and vital organs diseases in advanced stages. Patients for whom weekly follow-ups are non-feasible are also excluded.

CU patients who fulfilled the inclusion criteria were enrolled in the study after taking a written informed consent. At the baseline visit, a detailed history was taken to identify the severity of urticaria using three scoring systems, i.e., urticaria activity score (UAS),<sup>8</sup> urticaria severity score (USS),<sup>9</sup> and dermatology life quality index (DLQI).<sup>10</sup> After the clinical diagnosis of CSU was established, ASST was performed. Irrespective of the ASST results, all patients enrolled in the study received AST.

### ASST and AST Procedure

Before performing ASST, long-acting antihistamines were withdrawn 2 weeks prior and short-acting antihistamines & steroids were withdrawn 2 days prior to the procedure. For ASST, 2.5 ml of venous blood was collected in a sterile vacutainer and kept aside for 15 minutes to promote clot formation, and then centrifuged at 2500 rpm for 15 minutes to obtain autologous serum. A total of 0.1 ml of autologous serum and 0.1 ml of 0.9% normal saline (negative control) were injected using 1 ml insulin syringe with the bevel facing upward to develop a bleb at a gap of 5 cm over the right/left forearm on the uninvolved skin. After 30 minutes of injection, the results were read. A positive ASST was considered when the wheal induced by the autologous serum was >1.5 mm in diameter compared to the wheal induced by the normal saline. Irrespective of the ASST results, all patients were given AST.

For AST, 5 ml of the patient's venous blood was collected in a sterile vacutainer, and subsequently allowed to clot for 30 minutes. Later, the sample was subjected to centrifugation for 10 minutes at 3000 rpm at room temperature in a machine by R-8C laboratory centrifuge (REMI laboratory instruments, Mumbai, India).<sup>11</sup> A total of 2.5 ml of fresh serum obtained from the centrifugation was administered through deep intramuscular injection into the gluteal muscle or the upper arm.

All patients received this autologous serum injections for nine successive weeks (baseline and eight follow-up visits). At each weekly follow-up visit, UAS, USS, and DLQI assessments were conducted. Antihistamines were permitted once a day during the treatment period. Data analysis was conducted using statistical package for social sciences (SPSS) software version 21 (IBM Corp, New York, United States). Mean and percentages were calculated for data description.

## Results

Among 34 patients enrolled in the study, 22 were female (64.7%) and 12 were male (35.29%). The youngest patient enrolled in our study was aged 18 years and the oldest was aged 58 years, with a mean age of 34.04 years. Table 1 shows the age and sex distribution among ASST-positive and ASST-negative patients. The majority of subjects have had the disease for <12 months (25 subjects, 73.53%), with maximum duration of 6 years and minimum duration of 2 months. Figure 1 shows the duration of urticaria among our study subjects. Of the 34 subjects enrolled in the study, 24 (70.58%) had positive ASST, while 10 (29.41%) had negative ASST results. The average UAS among the 24 subjects with positive ASST results was 2.95 at baseline and improved to 0.29 at week 8 after AST. On the other hand, among the 10 subjects with negative ASST, the average UAS was 2.4 at baseline and improved to 0.4 at week 8 after AST.

Table 2 shows the subjects' UAS at baseline and after AST. Among the 24 subjects with positive ASST results, the average USS was 20.45 at baseline and improved to 1.83 at week 8 after AST.

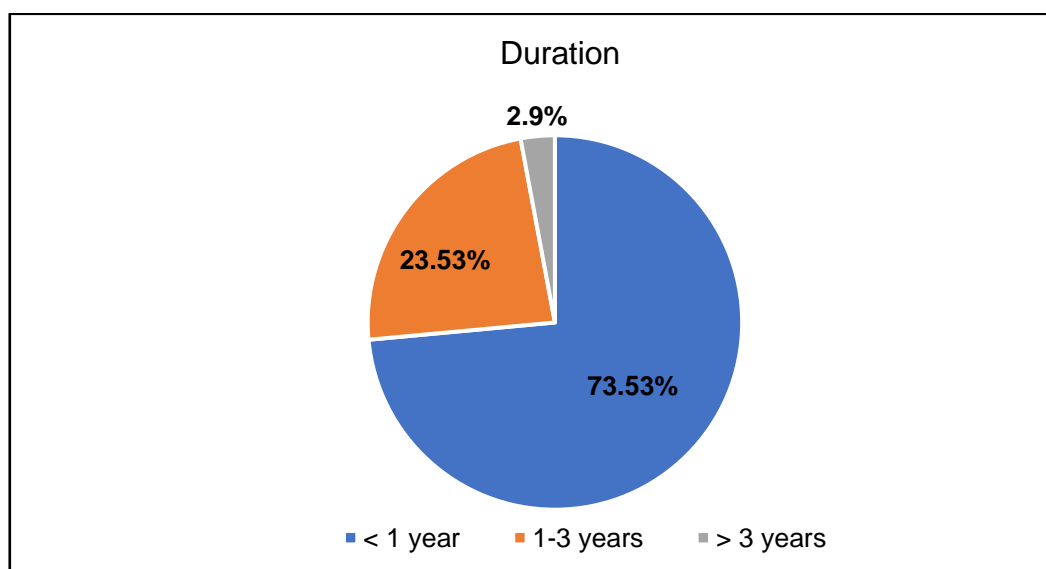
On the other hand, of the 10 subjects with negative ASST results, the average USS was 20.9 at baseline and improved to 2.8 at week 8 after AST. Table 2 also shows the subjects' USS at baseline and after AST. The average DLQI score among the 24 subjects with positive ASST was 6.6 at baseline and improved to 0.45 at week 8 after AST. On the other hand, of the 10 subjects with negative ASST results, the average DLQI score was 8 at baseline and improved to 0.6 at week 8 after AST.

The subjects' DLQI at baseline and after AST is also shown in Table 2. Data analysis was performed using Wilcoxon signed rank test, which showed improvement in UAS, USS, and DLQI scores among the study subjects before and after AST, irrespective of ASST results. This result was statistically significant (p-value <0.05). However, there was no statistically significant difference in UAS, USS, and DLQI scores at baseline (week 0) and after AST (week 8) between ASST positive and negative groups. So, all CSU subjects, irrespective of their ASST results, responded well to AST therapy. The limitations to our study is the small sample size and the lack of follow-up after the completion of AST.

**Table 1.** Comparison of Demographic Details between Autologous Serum Therapy Positive and Negative Groups

Variables	AST Positive (N=24)	AST Negative (N=10)
Age (in years)	31.6	36.4
Sex		
Male	9	3
Female	15	7

AST: autologous serum therapy



**Figure 1.** Duration of Urticaria Disease

**Table 2.** Comparison of Urticaria Activity Score, Urticaria Severity Score, and Dermatology Life Quality Index Before and After Treatment among the Autologous Serum Skin Test Positive and Negative Study Subjects

	Median (Q1-Q3)	Wilcoxon Signed Ranks Test	p-value
<b>ASST Positive (n=24)</b>			
<b>UAS</b>			
Week 0	3 (2-4)		
Week 8	0 (0-0.75)	-4.327	0.00001
<b>USS</b>			
Week 0	18.5 (11.25-28)		
Week 8	1 (0-3)	-4.288	0.00001
<b>DLQI</b>			
Week 0	6.5 (4-9.75)		
Week 8	0 (0-1)	-4.203	0.00002
<b>ASST Negative (n=10)</b>			
<b>UAS</b>			
Week 0	2 (1.75-3.25)		
Week 8	0 (0-0.5)	-2.641	0.008
<b>USS</b>			
Week 0	22 (15-25.25)		
Week 8	2 (0-4)	-2.821	0.005
<b>DLQI</b>			
Week 0	8.5 (6-10.25)		
Week 8	0 (0-0.25)	-2.703	0.007

ASST: autologous serum skin test; DLQI: dermatology life quality index; UAS: urticaria activity score; USS: urticaria severity score

## Discussion

CSU is a burden for the patients in terms of compliance, medical cost, and QoL. The goal of treatment in CSU is to control symptoms (wheals/angioedema) and to improve QoL. Despite being a chronic disease, CSU has low mortality rate and does not induce long-term organ damage.<sup>12</sup> The etiology of CSU is unidentified, and the pathogenesis is unclear. Mast cell degranulation, release of preformed mediators, and newly-synthesized mediators like histamine and leukotrienes result in hives/ wheals of urticaria. This is caused by the binding of autoantibodies to the high affinity receptor for IgE (FcεR1) on mast cells and basophils, which leads to their degranulation & release of mediators.<sup>13</sup>

First-line treatment for CSU is oral anti-histamines where the dose can be increased up to 4-fold until clinical improvement is achieved. Poor compliance

towards treatment (pill) necessitates a search for other treatment options that provide longer remission period. AST is an alternative therapy for CSU that acts by developing tolerance to histamine-releasing factors present in the serum of patients with autoimmune diseases. Intradermal injection of autologous serum in these patients elicits an immediate-type wheal-and-flare response, indicating the presence of a circulating histamine-releasing factor.<sup>14</sup> In a similar study by Darshana, et al.<sup>15</sup> ASST-positive group showed 69% improvement in urticaria total severity score (TSS) while ASST-negative group showed 61.5% improvement. At the end of follow-up, ASST-positive group had 77% improvement in TSS and ASST-negative group had 69% improvement.

In a study by Kumaran, et al.<sup>16</sup> 100 CSU patients with male-to-female ratio of 1:1.08 and a mean duration of illness of  $4.85 \pm 5.07$  years were evaluated. The comparison of positivity in cohorts

and controls for ASST and autologous plasma skin test (APST) was statistically significant, with higher positivity observed in cohorts compared to controls ( $p < 0.001$ ).

In a study by Surendran, et al.<sup>17</sup> two groups of 15 patients each with positive ASST results (50%) and negative ASST results (50%) were evaluated. In the group with positive ASST results, 8 patients (53%) had 31-60% improvement and 3 patients (20%) had 61-90% improvement. Among the 15 patients with negative ASST results, only 3 patients (20%) had 31-60% improvement and 4 patients (26.67%) had 61-90% improvement.

In a study by Godse, et al.<sup>18</sup> the efficacy of AST was studied by administering autologous serum through subcutaneous injection, which revealed an UAS reduction from 35.74 to 7 at the end of week 9; however, in the control group which received subcutaneous saline injection, there was no statistically significant UAS reduction. In a study by Talwar, et al.<sup>19</sup> significant UAS improvement was noted in both groups with positive and negative ASST results, from the baseline to week 9 (UAS improvement from 15.3 to 10.8 in ASST-positive patients and from 16.2 to 10.1 in ASST-negative patients).

A study by Vikramkumar, et al.<sup>20</sup> included 48 patients, 20 of whom (41.6%) had positive ASST results, while the remaining 28 (58%) had negative ASST results. No statistically significant difference in terms of mean age, gender, clinical morphology of individual wheals, duration, severity, systemic symptoms, angioedema, atopy, and association with other autoimmune conditions was noted between both groups, consisting of patients with and without antibodies.

In a study by Karn, et al. comparison between the ASST-positive and ASST-negative groups were made after AST, but no statistically significant improvement between both groups was observed.<sup>21</sup> In a meta-analysis by Chang, et al.<sup>22</sup> autologous whole blood therapy and AST were not effective in alleviating CSU symptoms compared to placebo treatment.

In a study by Parekh, et al.<sup>23</sup> 11 out of 39 patients had positive ASST results. The study also showed a significant decline in Urticaria TSS in both ASST-positive and ASST-negative groups following AST, despite there being no statistically significant difference between both groups. In a study by Yu, et al.<sup>24</sup> AST was administered to 66 ASST-positive patients, which resulted in a considerable improvement of disease activity and QoL. A total of

28% and 34% of patients had ASST negative results by week 9 and 21, respectively. In the patients who responded to AST, a decrease in the IgE-anti-IL-24 level was observed but not in the IgG-anti-IL-24 level. No changes were also observed in the basophil histamine release assay (BHRA) level.

In a case control study by Demirkan,<sup>25</sup> the ratio of ASST positivity between acute urticaria (AU) and CSU groups were 25.9% and 21.7%, respectively, which were higher than that of the controls (10.7%,  $p = 0.33$  for all groups). This suggests that ASST is insufficient to indicate autoimmunity. In a study by Paudel, et al.<sup>26</sup> among 114 CSU cases, 48.2% of patients who had higher QoL impairment had positive ASST results.

In a study by Park, et al.<sup>27</sup> 300 CSU patients were evaluated. A shorter duration of disease and more severe clinical features such as angioedema was observed in ASST or APST-positive patients but there was no significant difference between both groups with respect to autoantibodies positivity. Both ASST and ASPT groups showed consistent results in CSU.

In a study by Wannous, et al.<sup>28</sup> 50 CSU patients were studied with equal number of participants in both groups of ASST positive and negative results. 18 patients (36%) had an excellent response, 7 (14%) had a very good response, 11 (22%) had a good response, and 14 (28%) had no response. Although both ASST-positive and ASST-negative groups had good response towards AST, a significant difference was not observed between both groups.

In our study, we used two scoring systems (UAS and USS) to assess the disease severity and one scoring system (DLQI) to evaluate the impact of disease on the patient's QoL. The three scoring systems were used to avoid errors and strengthen the significance of results. Irrespective of ASST results, there was a statistically significant improvement in terms of UAS, USS and DLQI scores of the study subjects.

## Conclusion

In CSU patients who have limited control of their symptoms with oral antihistamines, AST is a prudent alternative treatment. ASST may sometimes yield a false-positive or negative result, highlighting the fact that this test is not a reliable indicator to start AST in CSU patients. Thus, AST is a potential and promising therapy in CSU, irrespective of ASST results. AST is a worthwhile

option for CSU patients, as it uses a substance originating from the patient's own serum, has very minimal side effects, and is cost-effective.

## Acknowledgments

None.

## Author Contributions

All authors act as the guarantor of the manuscript. SAS and AH are the main investigators of this study involved in study design and timeline. SAS, GJ, and DG participated in the conception & data acquisition. SAS and AH participated in data interpretation, writing of the study, data analysis and statistical analysis of the study.

## Conflict of Interests

No conflict of interest.

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