

Autologous Serum Skin Test and Autologous Whole Blood Injections to Patients with Chronic Urticaria: A Retrospective Analysis

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Background: Chronic urticaria (CU) is defined as widespread short-lived (< 24 h) itching maculopapular skin lesions with or without angioedema for more than 6 weeks for which a predominant physical cause must be excluded. The autologous serum skin test (ASST) has been shown to result in immediate hypersensitivity-type skin reactions in a subpopulation of CU patients and autologous whole blood (AWB) injection has been used as one of treatment modalities.

Objective: To evaluate the relationship of a panel of laboratory examinations to the positivity of ASST and to evaluate the efficacy of AWB injection in the treatment of ASST (+) and ASST (-) CU patients.

Methods: A retrospective chart review was performed on CU patients who were subjected to ASST and received AWB injection therapy. We assessed the therapeutic effects of 8 weekly AWB injections in ASST (+) and ASST (-) CU patients by using urticaria activity score (UAS).

Results: Thirty-seven patients were analysed. There was no significant differences in number of patients, sex distribution, age, smoking vs. non-smoking, exacerbation of urticarial symptoms due to stress, and UAS between ASST (+) and ASST (-) CU patients at baseline. All patients presented normal complete blood count/differential count, and ANA. Anti-microsomal antibody was positive in 3/15 (20.0%) ASST (+) patients and in 2/22 (9.0%) ASST (-) patients ($P = 0.6$). The 8-week course of AWB injection was well tolerated. ASST (+) patients, but not ASST (-) patients, showed significantly reduced CU activity.

Conclusion: 8 of the 9 ASST (+) patients with CU responded to treatment with AWB injection. Only 2 of the 8 ASST (-) patients showed response to the treatment. Others were having no effects or exacerbation of the urticarial symptoms. Further studies utilizing larger number of patients, longer follow-up periods, and different amount of autologous serum injection may better define the clinical efficacy of autologous serum injection for the treatment of chronic urticaria with positive ASST. (Dermatol Sinica 27: 27-36, 2009)

Key words : Chronic urticaria, Autologous serum skin test, Autologous whole blood injection

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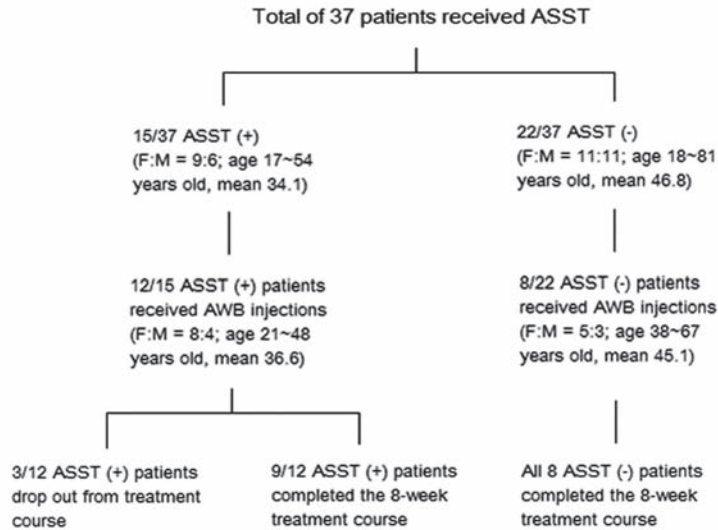
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**Fig. 1**

Study design, stratification of patients.

INTRODUCTION

Chronic urticaria (CU) is defined as widespread short-lived (< 24 h) itching maculopapular skin lesions with or without angioedema for more than 6 weeks and is divided into 2 major subgroups, chronic autoimmune urticaria (CAU, 45%) and chronic idiopathic urticaria (CIU, 55%), which have a combined incidence in the general population of 0.5%. Cases with a predominant physical cause must be excluded from this definition.¹

There is circumstantial evidence to support an autoimmune basis for a subset of patients with CU. First, the presence of circulating functional histamine-releasing immunoglobulin G (IgG) autoantibodies directed toward the α -chain of the high-affinity IgE receptor (Fc ϵ RI α), present on mast cell and basophil membranes, and, less frequently, of IgG directed toward the IgE molecule, capable of activating mast cell and basophil degranulation, suggests the autoimmune etiopathogenesis of the disease in a subset of patients.²⁻⁹ Hide *et al.*⁵ reported that an intracutaneous injection of serum, i.e., the autolo-

gous serum skin test (ASST), resulted in immediate hypersensitivity-type skin reactions in a subpopulation of CU patients. The ASST has been reported to be positive in 41%~67% of all CU patients.^{5, 10-12} The ASST is an in vivo-validated test, performed by injecting a patient's own serum into the skin in order to mimic CU autoreactivity. A positive reaction to the ASST, reflecting the presence of factors capable of degranulating mast cells, is regarded as a reliable in vivo diagnostic test in CU patients.⁵

Second, a series of studies has suggested an autoimmune cause of chronic urticaria in its association with autoimmune thyroiditis. Of 182 patients with CU in 1 report, 36 were found to have thyroid microsomal autoantibodies and/or an abnormal plasma thyroid-stimulating hormone level; in addition, there was marked cosegregation of these patients with those with a positive ASST indicative of CU autoantibodies.¹³

As circulating histamine-releasing factors may participate in the pathogenesis of CU, autohemotherapy or an autologous whole blood (AWB) injection is used as 1

of the treatment modalities. In a randomized, placebo-controlled study conducted by Staubach *et al.*,¹⁴ ASST (+) CU patients showed significant improvement after an AWB injection, clearly exhibiting responses which differed from those of AWB-treated ASST (-) CU and placebo-treated patients.

The ASST examination is routinely performed with permission from patients with established CU in our institute who fulfill the criteria (see method) since 2006. They may subsequently receive AWB injection therapy. To our knowledge, there are no published data regarding the efficacy of AWB injections in the treatment of ASST (+) and ASST (-) CU patients in the Taiwanese population. We retrospectively analyzed patients with established CU who were subjected to ASST and received AWB injection therapy in our medical institute from 2006 to 2008. In all cases, clinical findings, laboratory findings, and urticaria activity score (UAS) data were collected. This retrospective review was intended to evaluate the relationship of a panel of laboratory examinations with the positivity of ASST and to evaluate the efficacy of AWB injections in treating ASST (+) and ASST (-) CU patients.

MATERIAL AND METHOD

Patients:

In total, 37 CU patients (with ≥ 6 weeks duration of disease) were subjected to skin tests with their own serum (Fig. 1). Fifteen of them exhibited a positive ASST reaction. Twelve (8 females aged 36.6 range from 21 to 48 years) of these 15 ASST(+) patients were compared with eight ASST(-) CU patients (5 females aged 45.1 range from 38 to 67 years) for differences in clinical parameters and were then treated with repeated injections of AWB. Each patient provided written informed consent. Patients in following conditions: chronic urticaria due to predominantly physical causes, pregnancy, lac-



Fig. 2

A positive autologous serum skin test, at 30 min. (Injections: saline (top); autologous serum (bottom))

tation or risk of pregnancy without medically approved contraception, severe systemic diseases, as well as anticoagulation or systemic corticoid therapy were not subjected to treatment. All patients underwent the following evaluation consisting of a clinical assessment including routine blood screening (complete blood count, CBC), differential count (DC), the erythrocyte sedimentation rate (ESR), thyroid function test, anti-microsomal antibody (AMA), and anti-nuclear antibody (ANA) for undetected systemic illnesses.

Autologous Serum Skin Test:

Venous blood was taken and serum was obtained by centrifugation (500 g, 10 min). An intradermal test with 0.05 ml of fresh, undiluted, autologous serum and a saline control was performed and evaluated 30 min later. A difference of > 1.5 mm between the wheal diameter elicited by the autologous serum and that by saline was considered a positive ASST. Intervals of at least 5 cm were left between the injection sites, and areas known to have been involved in spontaneous whealing during the last 24 h were avoided¹⁵ (Fig. 2). Short-acting antihistamines were with-

Table. 1 Description of Scoring System

Symptoms	Scoring value			
	0	1	2	3
Wheal number (daily average)	None	<10	10-50	>50
Wheal size (average diameter)	None	<1 cm	1-3 cm	>3 cm
Wheal duration (average, h)	None	<1 h	1-24 h	>24 h
Pruritus intensity (daily average)	None	Mild	Moderate	Severe
Pruritus duration (average, h)	None	<1 h	1-24 h	>24 h
Erythema number (daily average)	None	<10	10-30	>30
Erythema size (average diameter)	None	<1 cm	1-3 cm	>3 cm
Erythema duration (average, h)	None	<1 h	1-24 h	>24 h
Angioedema number (daily average)	None	1	2	3
Angioedema size (average diameter)	None	<1 cm	1-3 cm	>3 cm
Angioedema duration (average, h)	None	<1 h	1-24 h	>24 h

Modified from references by O'Donnell *et al.*¹⁶ and Grattan *et al.*¹⁷

drawn at least 2 days prior to the skin tests. No subject had taken long-acting antihistamines or an immunosuppressant for at least 2 weeks before testing.

AWBI Method:

The treatment duration and method of administration were the same as follow: (1) a run-in-period of 1 week to record baseline symptoms and (2) 8 weekly intramuscular gluteal injections (alternating sides; blood was taken immediately before the reinjection from a cubital vein; blood was not subjected to anticoagulation or other treatment). This frequency/number of injections and the volume of the injections (first injection: 2.5 ml; injections 2~8: 3~5 ml) were chosen based on earlier reports from the literature which had shown that this regimen can be effective in treating CU patients.¹⁴

Clinical Assessment:

CU patients recorded the expression of urticarial symptoms using score values from 1 to 3 and the need for rescue medication (the amount of antihistamine required per day). The UAS, based on the UAS used by O'Donnell *et al.*¹⁶ and Grattan *et al.*,¹⁷ was

calculated in weeks 0 (before treatment), 8 (immediately after end of treatment), and 12 (4 weeks after the end of treatment). Briefly, scores of wheal number, size, and duration; pruritus intensity and duration; erythema number, size, and duration; and angioedema number, size, and duration at weeks 0, 8, and 12 were each totaled to obtain the UAS (range, 0~33) (Table 1).^{16,17}

Outcome Measures:

Outcome measures included (1) an improvement in urticarial symptoms and (2) the requirement for antihistaminic rescue medication before as well as 8 and 12 weeks after initiation of treatment.

Statistical Analysis:

Statistical analysis was performed using the software package, Statistical Analyzing System 8e (SAS8e). Associations between categorical groups were assessed using Chi-squared test or Fisher's exact test as a descriptive analysis of patients, and the Wilcoxon signed-rank sums test was used for paired data. Between-group comparisons of before and after treatment disease activity (the UAS) were performed using the signed-

Table. 2 Baseline Demographic Data and Disease Characteristics of All Patients Who Received ASST

	ASST (+)	ASST (-)
Total Patients	15	22
Ages, years		
Range	17~54	18 ~ 81
Mean	34.1	46.8
Sex, no. (%)		
Male	6 (40)	11 (50)
Female	9 (60)	11 (50)
Duration of disease, years	1.36	1.22
Positivity of AMA, no. (%)	3 (20)	2 (9)

Table. 3 Urticaria Activity Score of ASST (+) and ASST (-) Patients with AWB Injection Treatment at Week 0

	ASST (+) CU patients	ASST (-) CU patients	Statistical significance
UAS, range: 0-33			
Urticaria activity score	13.5 ± 3.5	12.4 ± 4.2	n.s.
Wheal (scores), range: 0-3			
Number	1.6 ± 0.5	1.6 ± 0.6	n.s.
Size, average diameter, cm	2.0 ± 0.6	2.0 ± 0.7	n.s.
Duration, average, h	1.6 ± 0.5	1.5 ± 0.5	n.s.
Erythema (scores), range:0-3			
Number	1.4 ± 0.7	1.1 ± 1.0	n.s.
Size, average diameter, cm	1.4 ± 0.7	0.6 ± 0.5	n.s.
Duration, average, h	1.4 ± 0.6	1.1 ± 0.8	n.s.
Angioedema (scores), range: 0-3			
Number	0.2 ± 0.4	0.3 ± 0.6	n.s.
Size, average diameter, cm	0.3 ± 0.7	0.3 ± 0.6	n.s.
Duration, average, h	0.3 ± 0.6	0.1 ± 0.3	n.s.
Pruritus (scores), range: 0-3			
Intensity, daily average	1.8 ± 0.9	2.1 ± 0.8	n.s.
Duration, average, h	1.4 ± 0.6	1.5 ± 0.5	n.s.

All data are presented as mean ± SEM. n.s. = Not significant.

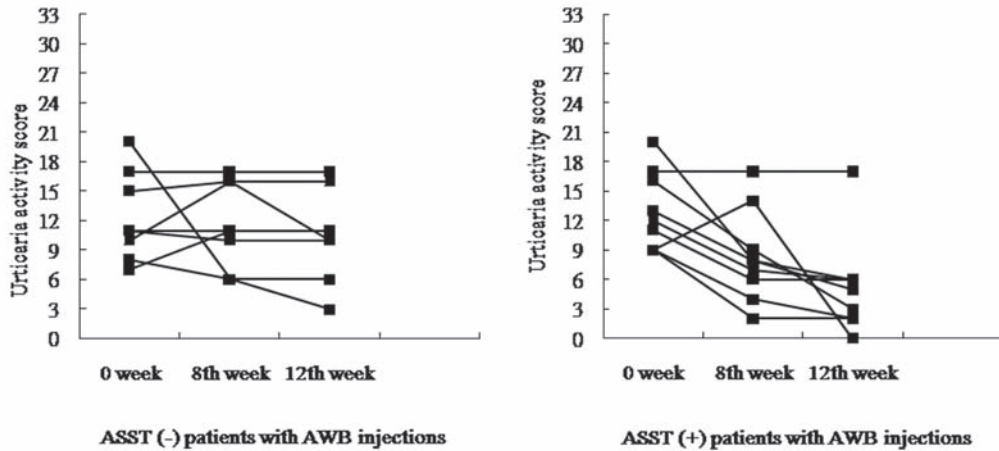
rank test. A *p* value of < 0.05 was considered to indicate a statistically significant difference.

RESULTS

The demographics and clinical characteristics of all patients at baseline are presented in Table 2, 3.

Clinical Profiles of ASST (+) and ASST(-) CU Patients

The prevalence of positive ASST in our CU patients was 40% (15/37). Among those 15 ASST (+) patients, 12 received AWB injections and 9 completed the full course of treatment (8 weeks). Three of the 12 patients terminated treatment early as a result of lim-

**Fig. 3**

ASST (+) CU patients exhibit improvement of urticarial symptoms after treatment with AWB. Individual changes of weekly UAS scores in ASST (+) or ASST (-) patients treated with 8 weekly intramuscular injections of AWB.

ited subjective treatment response after the 2nd or 3rd (2 patients) injection, respectively. Among 22 ASST (-) patients, 8 received AWB injections, and all 8 completed the full treatment. No significant differences were detected in the gender distribution, age, smoking vs. non-smoking, exacerbation of urticarial symptoms due to stress, or UAS at the baseline.

Laboratory Analyses

All patients presented a normal CBC/DC, and ANA. There were no significant differences among ASST (+) and ASST (-) patients in terms of those laboratory examinations. AMA was positive (>1:100X) in 3 of 15 (20.0%) ASST (+) patients and 2 of 22 (9.0%) ASST (-) patients ($p = 0.6$). The ESR was above the normal limit in 2 of 15 (13.3%) ASST (+) patients and 5 of 22 (22.7%) ASST (-) patients ($p = 0.7$). One ASST (+) patient was diagnosed as having hyperthyroidism under regular medication control. All ASST (-) patients appeared to have a normal thyroid function test.

Per-Protocol Analysis of Repeated Injections

of AWB Significantly Reduced Disease Activity in ASST (+) CU patients, but Not in ASST (-) CU Patients (Fig. 3, 4)

Eight of 9 ASST(+) CU patients showed significant improvement in the UASs after repeated injections of AWB (week 0 vs. 12, $p = 0.009$). Only 2 of 8 ASST(-) CU patients exhibited reduced UASs after treatment. There was no significant improvement of the average UAS after treatment in ASST (-) CU patients ($p = 0.69$). When we compared treatment groups (ASST (+) and ASST (-) CU patients) for average changes in disease activity, we found that ASST(+) patients showed markedly improved UASs after AWB therapy ($p = 0.02$).

AWB injections were found to be well tolerated and safe. None of the patients enrolled reported serious adverse events due to the treatment. One patient reported experiencing an irregular menstrual cycle and hypermenorrhea during the treatment period; however, after consultation with a gynecologist, she continued with the AWB injections and completed the treatment course. A significant improvement in the UAS was seen in this patient. None of the patient experienced

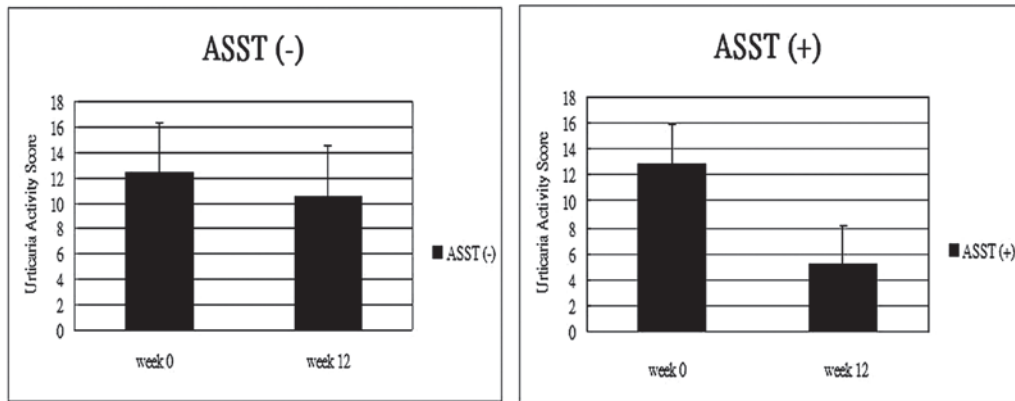


Fig. 4

Urticaria Activity Score (week 0 v.s week 12) of 9 ASST (+) and 8 ASST (-) patients who completed the 8-week AWBI treatment course.

hematoma or soreness at the injection site, or allergic reactions. There was no increased incidence of infections. There were no other serious adverse effects throughout the treatment course.

Antihistaminic Rescue Medication

This retrospective review failed to demonstrate any correlation between the requirement for antihistaminic rescue medication before and after AWB injections in ASST (+) or ASST(-) patients due to different patients' adherence characteristics to the physician's medical orders. However, in general, according to the medical records and telephone interviews of patients, 12 weeks after the initiation of therapy, most of the ASST (+) patients treated with AWB required less of the antihistaminic rescue medication than they had taken before the therapy. One ASST (+) patient is still free from CU symptoms and antihistaminic rescue 8 months after AWB therapy. Such an observation was not seen among ASST (-) patients who received AWB injections.

DISCUSSION

Chronic urticaria, a common dermatosis characterized by spontaneously occurring

short-lived, itchy wheal and flare-type skin reactions which is divided into two major subgroups, CAU and CIU.¹ Some suggests that CU may be caused by a large number and variety of distinct conditions including intolerance to food or drugs as well as infectious and autoimmune diseases.^{18,19}

There is now strong evidence of an autoimmune basis for the disease in some patients, and circulating functional histamine-releasing autoantibodies reactive against either the α subunit of the high affinity IgE receptor (Fc ϵ RI α) or IgE have been identified in about 1/3 of patients with CU.^{3,5,6,11,20} Autoantibodies in patients' serum can be detected by serum-induced histamine release from the basophils of healthy donors, or by Western blot analysis.^{3,6,11} At present, these tests are only carried out in a few specialist laboratories, are time-consuming to perform, and are not available to the majority of clinicians.¹⁵ An intradermal injection of autologous serum in some patients with CU produces a wheal and flare response. This simple observation suggests the evidence of circulating histamine-releasing factors, for example, functional anti-Fc ϵ RI α , in the serum of these patients.⁵ Currently, ASST, a nonspecific screening test which evaluates

the presence of serum histamine-releasing factors of any type, not just histamine-releasing autoantibodies, is generally recognized as a screening test for autoantibodies against the IgE receptor.²¹

Given that a subset of CU patients who exhibit a positive ASST may benefit from AWB injections,¹⁴ we have performed the ASST regularly on CU patients who visit our outpatient clinic since 2006; the patient must meet the criteria described in method prior to the examination. AWB injections are given as a treatment option after a thorough explanation of the mechanisms and treatment protocol. We chose the dosing regimen based on earlier reports from the literature which showed that this regimen can be effective in treating CU patients.

The statistical analysis showed no significant differences in the gender distribution, age, smoking vs. non-smoking, exacerbation of urticarial symptoms due to stress, a panel of laboratory examinations, and the UAS at the baseline.

Thyroid microsomal autoantibodies and/or an abnormal plasma thyroid-stimulating hormone level are reported to have marked cosegregation of these patients with those with a positive ASST;¹³ however, such a phenomenon was not observed in our patient group (3 of 15 ASST (+) patients), most likely due to the limited number of patients examined.

Confirming previous reports, about 1/3 of CU patients (15 of 37, 40%) were found to exhibit positive ASST responses.^{5, 10-12, 14} The treatment regimen of AWB injection therapy for 8 weeks was well tolerated by our patients. The response to 8 weekly AWB injections in the ASST (+) population was good, and the result was similar or better in comparing to the previous reports.^{14, 22} Eight of 9 ASST (+) patients who completed the full AWB injection treatment course showed significant reduced CU activity. Only 2 of

8 ASST (-) patients showed a response to the treatment. Others exhibited no effects or exacerbation of the urticarial symptoms. Most of the ASST (+) patients who received AWB therapy started to show improvement of urticaria disease activity from week 3~4 after initiation of the treatment. Furthermore, in an average of 8 months follow-up period, most of the these patients required less of the antihistaminic rescue medication than they had taken before the therapy. One ASST (+) patient is still free from CU symptoms and antihistaminic rescue 8 months after AWB therapy. These findings suggest that ASST (+) CU patients can benefit from a simple, inexpensive, effective, potentially curative, and safe therapy with AWB injections.

Due to the fact that this is a retrospective chart analysis, we believe that it's suboptimal that we did not include the 3 of the 12 ASST (+) patients who terminated treatment early as a result of limited subjective treatment response in our disease activity statistical analysis. However, if we consider the treatment to be non-effective in these 3 patients and compare treatment groups (ASST (+) and ASST (-) CU patients) for average changes in disease activity, the *p* value is 0.07.

We report the results of a single medical center clinical experience with the use of AWB injections in treating CU patients. We have shown AWB injections to be safe and effective in treating symptoms of ASST (+) CU patients. The study was inherently limited by the small sample size and short follow-up period. Given the tolerability of the AWB injections and the need for a safe therapy for the long-term control of urticarial symptoms in CU patients, further cohort-controlled studies utilizing larger numbers of patients, longer follow-up periods, and different amounts of the autologous serum injection may better define the clinical efficacy of autologous serum injections for treating

ASST (+) CU patients.

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探討自體血清注射試驗及自體全血注射治療於慢性蕁麻疹病患中所扮演的角色：一個回顧性分析

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背景：慢性蕁麻疹（chronic urticaria）定義為持續六周以上反覆短暫性（< 24小時）皮膚膨疹，可伴隨厲害的癢感或血管水腫（angioedema）。一般此定義必須先排除主要以物理性蕁麻疹為表現的病患。許多的大型研究證實有一部分慢性蕁麻疹病患體內存在著特殊的自體抗體，這些自體抗體會促使肥大細胞及嗜鹼性白血球分泌組織胺，進而誘發蕁麻疹。

目的：我們回顧病歷記載，試著去探討自體血清注射試驗（autologous serum skin test）之結果與生化、血液、血清免疫檢查的相關性及是否與自體全血注射（autologous whole blood injection）之反應結果有相關。

方法：經由病患主觀蕁麻疹嚴重程度表來評估其改善程度。我們比較自體血清注射為陽性及陰性的病患在接受完為期八周（每周一次）的自體全血注射治療的結果。

結果：大多數（8/9）自體血清注射試驗呈陽性反應的病患在接受完一個療程的全血注射後其蕁麻疹的嚴重程度大幅減低，呈陰性反應的族群中並沒有看到類似的效果，兩者差別具統計學上顯著差異（ $p = 0.02$ ）。於一些生化、血液、及血清免疫的檢查，自體血清注射試驗呈陽性或陰性反應的病患中並沒有重大區別。

結論：大多數自體血清注射試驗呈陽性反應的病患在接受完一個療程的全血注射後其蕁麻疹的嚴重程度大幅減低，呈陰性反應的族群中並沒有看到類似的效果，然而，此研究受限於樣本數少及治療後的追蹤期較短。未來，需要納入更多的患者、追蹤更長時間且使用不同的自體全血注射劑量，始能對自體血清注射試驗呈陽性反應的蕁麻疹病患訂定一個更明確的治療方針。（中華皮誌：27: 27-36, 2009）

