

## Report

# Influence of narrowband UVB irradiation of serum on autologous serum skin test results

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### Abstract

**Background** Autologous serum skin test (ASST) is an *in vivo* test to demonstrate circulating endogenous proinflammatory or wheal-inducing factors in urticaria patients. While ASST was negative in a patient with solar urticaria and in a patient with localized heat urticaria, test results turned to positive when performed with visible light-irradiated and heated serums, respectively. These data suggest that some factors and/or antibodies in serum may be activated or inhibited with different factors. We aimed to investigate whether or not ASST results change when performed with narrowband ultraviolet B (UVB)-irradiated serum.

**Materials and methods** Seventy-seven patients with chronic idiopathic urticaria underwent ASST twice in consecutive days. The second ASST was performed with narrowband UVB-irradiated serum. The results of the first and second ASSTs were compared.

**Results** The first ASST results were positive in 46 (59.7%) patients. ASST results reversed oppositely in 36 (46.8%) patients, turned from negative to positive in 19 patients, and turned from positive to negative in 17 patients when performed with narrowband UVB-irradiated serum.

**Conclusion** Our results suggest that narrowband UVB may have modifying effects on some wheal-inducing factors and/or antibodies. On which factors in serum narrowband UVB has effects may be investigated.

### Introduction

The autologous serum skin test (ASST) is an *in vivo* screening test for functional autoantibodies in chronic urticaria (CU). It does not prove the presence of antibodies, it rather shows histamine-releasing properties of the tested serum.<sup>1</sup> It was observed that while the ASST result was negative in a patient with localized heat urticaria, the result turned to positive when ASST was performed with heated serum, indicating that the patient's serum may have contained some urticaria-inducing factors that can be modified by heat.<sup>2</sup> Additionally, visible light-irradiated serum induced a positive reaction in an ASST-negative (ASST-) solar urticaria patient, which was inhibited with exposition of inhibition spectrum light.<sup>3</sup> All these data suggest that some factors and/or antibodies in serum may be activated or inhibited with some factors. It is already known that ultraviolet (UV) light has some proinflammatory effects. Human mast cells may be affected by UV light directly by altering the cell's potential of mediator release.<sup>4</sup> The efficacy of narrowband UVB in the treatment of CU was also established clinically by different

authors, but no difference was found in treatment response between ASST positive (ASST+) and ASST- patients.<sup>5,6</sup> In this study, we aimed to investigate whether narrowband UVB irradiation of serum has any effect on ASST results.

### Materials and methods

This study was conducted at the Department of Dermatology, Faculty of Medicine, Pamukkale University between November 1, 2009, and December 31, 2010. Patients with chronic idiopathic urticaria aged 18 years old or older were enrolled in the study. Urticaria activity was estimated according to the number of wheals present at the time of assessment and scored as follows: 0, no wheals; 1, 1–10 small (<3 cm in diameter) wheals; 2, 10–50 small wheals or 1–10 large wheals; 3, >50 small wheals or 10–50 large wheals; and 4, virtually covered with wheals.<sup>7</sup> Severity of itching was stated as no itch (0), mild (1), moderate (2), and severe (3). All patients were asked for physical urticaria in detail and underwent investigations including full blood count, biochemical profile, serological markers for hepatitis, thyroid function tests,

antithyroid antibodies, urea breath test for *Helicobacter pylori*, and ASST. Patients were excluded if they had received antihistamines, systemic steroids, or immunosuppressive agents within the last seven days, four weeks, and three months, respectively. All patients underwent ASST twice in consecutive days. ASST was performed according to the European Academy of Allergy and Clinical Immunology-Global Allergy and Asthma European Network task force report.<sup>8</sup>

Venous blood was collected into sterile 8.5-ml BD Vacutainer<sup>™</sup> blood collection tubes (no additive; Becton, Dickinson, Franklin Lakes, NJ, USA) without accelerator or anticoagulant. Blood was allowed to clot at room temperature for 30 minutes, then separation was performed with a bench centrifuge at a relative centrifugal force of 500 *g* (expressed in units of gravity) for 10 minutes. Fresh undiluted serum (0.05 ml) and 0.9% sterile saline (negative control) were injected with an interval of at least 3 cm into the volar aspect of the forearm, on which no spontaneous wheals were present in the previous 48 hours. The result was evaluated after 30 minutes and judged to be positive when a serum-induced weal with a diameter of 3 mm greater than the negative control, surrounded by erythema, was present. Patients were recalled the following day, and serum was collected in the same way as described above. The serum was exposed to 250 mJ/cm<sup>2</sup> UVB (Philips TL 100W/01, Hamburg, Germany; main emission between 310 and 315 nm, maximum emission at about 311 nm). The second ASST was performed with narrowband UVB-irradiated serum. The results of the first and second ASSTs were compared.

This study was approved by the local ethics committee, and all subjects gave informed consent. The chi square test, Student's *t*-test, and Fisher's exact test were used for statistical analyses with SPSS software (version 13.0; SPSS, Chicago, IL, USA). *P*-values < 0.05 were considered significant.

## Results

Seventy-seven patients (49 female, 28 male) were included in the study. The first ASST results were identified as

**Table 2** The relation between clinical aspects and ASST results

|                          | Urticarial activity score |    |   |   |   | Pruritus severity |   |    |    |
|--------------------------|---------------------------|----|---|---|---|-------------------|---|----|----|
|                          | 0                         | 1  | 2 | 3 | 4 | 0                 | 1 | 2  | 3  |
| ASST+ patients, <i>n</i> | 25                        | 13 | 5 | 3 | 0 | 12                | 6 | 13 | 15 |
| ASST- patients, <i>n</i> | 17                        | 13 | 1 | 0 | 0 | 9                 | 9 | 3  | 10 |
| <i>P</i> -value          | >0.05                     |    |   |   |   | >0.05             |   |    |    |

ASST, autologous serum skin test.

positive in 46 (59.7%) patients. The demographic characteristics of ASST+ and ASST- patients were comparable, as demonstrated in Table 1. Urticaria activity and pruritus severity did not differ between ASST+ and ASST- patients (*P* > 0.05; Table 2). It was observed that ASST results reversed oppositely in 36 (46.8%) patients, turned from negative to positive in 19 patients, and turned from positive to negative in 17 patients when performed with narrowband UVB-irradiated serum (Fig. 1; Table 3).

## Discussion

The ASST is an *in vivo* test that assesses autoreactivity. Autoreactivity is characterized by an itchy wheal and flare response to intradermally injected factors in autologous serum acting either indirectly through the release of mediators from cutaneous mast cells or other cells or directly on the microvasculature of skin. Autoreactivity does not define autoimmune urticaria.<sup>8,9</sup>

The frequency of ASST+ results range from 4.1% to 76.5% for adult patients. Patient selection, disease severity, methodology, and response interpretation might lead to these differences. No association was found between ASST response and gender and age by different authors. However, one study reported a higher prevalence of ASST positivity in women than in men. There are also controversial

**Table 1** The demographic characteristics of ASST+ and ASST- CU patients

|                                                  | ASST+ patients <i>n</i> = 46 | ASST- patients <i>n</i> = 31 | <i>P</i> |
|--------------------------------------------------|------------------------------|------------------------------|----------|
| Age, mean $\pm$ SD (min-max, years)              | 38.3 $\pm$ 15.0 (16-76)      | 40.2 $\pm$ 13.2 (17-65)      | >0.05    |
| Female, <i>n</i> (%)                             | 28 (60.9%)                   | 21 (67.7%)                   | >0.05    |
| Disease duration, median (min-max, years)        | 2.7 $\pm$ 3.8 (0.2-20)       | 2.2 $\pm$ 3.1 (0.2-16)       | >0.05    |
| Patients with allergic diseases, <i>n</i> (%)    | 3 (6.5%)                     | 2 (6.5%)                     | >0.05    |
| Patients with thyroid diseases, <i>n</i> (%)     | 3 (6.5%)                     | 3 (9.7%)                     | >0.05    |
| Patients with physical urticaria, <i>n</i> (%)   | 26 (56.5%)                   | 16 (51.6%)                   | >0.05    |
| Patients with thyroid antibodies, <i>n</i> (%)   | 8 (17.4%)                    | 7 (22.6%)                    | >0.05    |
| Urea breath test-positive patients, <i>n</i> (%) | 14 (30.4%)                   | 11 (35.5%)                   | >0.05    |

ASST, autologous serum skin test.

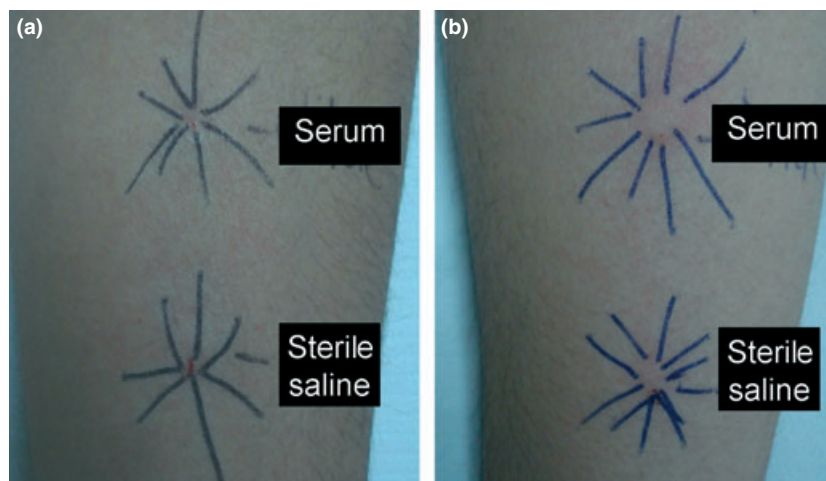


**Figure 1** (a) A patient whose first ASST result was negative. (b) The ASST result turned to positive when performed with narrow-band UVB-irradiated serum (the induration borders are marked)

**Table 3** The results of ASSTs

| Results        | Second ASST (n) |          |       |
|----------------|-----------------|----------|-------|
|                | Positive        | Negative | Total |
| First ASST (n) |                 |          |       |
| Positive       | 29              | 17       | 46    |
| Negative       | 19              | 12       | 31    |
| Total          | 48              | 29       | 77    |

ASST, autologous serum skin test.

results regarding the association between ASST positivity and disease severity, itch severity, and disease duration.<sup>8</sup>

In our study, ASST was positive in 59.7% of the patients, compatible with other reports. We did not find any difference between ASST+ and ASST- patients in terms of age, gender, disease severity, disease duration, pruritus severity, coexistence of physical urticaria, coexistence of thyroid and allergic diseases, antithyroid antibodies, and urea breath test positivity.

It was experienced that performing several procedures on patient's serum may enhance the ASST results. While ASST was negative in a patient with heat urticaria, the second ASST performed with heated serum resulted in a positive reaction.<sup>2</sup> In addition, the ASST result of a patient with solar urticaria turned to positive when performed after exposing serum to visible light, as well.<sup>3</sup> These observations indicate that some factors and/or antibodies in serum may lose or acquire wheal-inducing activity when exposed to different factors. According to our results, UVB may be one of these factors, as ASST results turned from negative to positive or just the opposite, from positive to negative, in 46.8% of the patients in our study.

Phototherapy has been successfully used in solar urticaria patients showing urticarial reaction to UV light exposure in different wavelengths.<sup>10</sup> PUVA was already shown to be more effective than H<sub>1</sub>-antihistamines in solar urticaria.<sup>11</sup> Narrowband UVB was found to be an effective treatment option for symptomatic dermographism, as well.<sup>12</sup> It was observed that UV irradiation of mast cells led to the inhibition of anti-IgE-stimulated histamine release from mast cells.<sup>4</sup> Phototherapy is also accepted as an additive therapy in CU.<sup>13</sup> In a retrospective analysis of 94 CU patients, it was seen that 72% of phototherapy courses produced clearance or improvement.<sup>6</sup> Engin *et al.* demonstrated that narrowband UVB combined with antihistamine reduced urticaria activity significantly more than antihistamine alone. They did not find any difference in treatment response between the ASST+ and ASST- patients.<sup>5</sup> However, it is not known yet whether the patients whose ASST turn from positive to negative when performed with narrow band UVB-irradiated serum derive more benefit from narrowband UVB therapy or contrarily the patients whose ASST turn from negative to positive drive no benefit from narrowband UVB therapy or even experience exacerbation of their disease. This may be influential in choosing the ideal line of treatment. Further investigations are also required to define the nature and functional properties of the serum factors before and after irradiation to detect the effect of narrowband UVB and which factors were activated or inhibited by it.

In conclusion, narrowband UVB may have modifying effects on wheal-inducing factors or antibodies. On which factors in serum narrowband UVB has effects may be the subject of further studies. The clinical significance of this finding may also be investigated.

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