

Autologous serum skin test response in chronic spontaneous urticaria and respiratory diseases and its relationship with serum interleukin-18 level

Emel Kurt · Ayse Aktas · Kurtulus Aksu ·
Metin Keren · Ali Dokumacioglu ·
Christopher H. Goss · Ozkan Alatas

Received: 29 December 2010 / Revised: 16 February 2011 / Accepted: 24 February 2011
© Springer-Verlag 2011

Abstract Autologous serum skin test (ASST) is mostly used in chronic spontaneous urticaria (CSU) to show autoreactivity. Interleukin-18 (IL-18) has also been shown to be involved in autoimmune conditions. To investigate the role of autoreactivity assessed by ASST in CSU and respiratory diseases and to investigate whether this auto-reactive state is related to IL-18 level or other clinical covariates. Fifty-five patients with CSU (mean age: 40.3 ± 12.3 years), 70 patients with persistent asthma (mean age: 43.7 ± 9.6 years), 21 patients with seasonal allergic rhinitis (SAR) (mean age: 35.5 ± 11.8 years) and 20 normal controls (mean age: 37.7 ± 9.8) were included. All subjects underwent a laboratory examination and skin prick test. ASST was performed and serum IL-18 levels were measured in all subjects. Positive response to ASST and serum IL-18 levels were higher in CSU patients than those with respiratory diseases (asthma and SAR)

($P = 0.034$ and 0.002 , respectively) and normal controls ($P = 0.004$ and 0.031 , respectively). Considering all patients, IL-18 levels were higher in patients with positive ASST (301.8 ± 194.4 vs. 241.8 ± 206.3 pg/ml, $P = 0.036$) than ASST negative patients. ASST response was associated with disease severity in CSU ($P = 0.037$) and asthma patients ($P = 0.001$). Multivariate analysis showed that positive response to ASST was significantly associated with diagnosis of CSU (OR: 3.13, 95% CI: 1.25–7.87) and female gender (OR: 3.98, 95% CI: 1.19–13.38). ASST response could be related with activity of the disease. A positive ASST response found in respiratory diseases patients suggests that it may occur as a result of some inflammatory events during the diseases' process.

Keywords Autologous serum skin test · Allergy · Asthma · IL-18 · Urticaria

A. Aktas, K. Aksu and M. Keren equally contributed to the study.

E. Kurt (✉) · A. Aktas · K. Aksu · M. Keren
Department of Allergy, Eskişehir Osmangazi University,
Eskişehir, Turkey
e-mail: dremelkurt@yahoo.com

A. Dokumacioglu · O. Alatas
Department of Clinical Biochemistry,
Eskişehir Osmangazi University, Eskişehir, Turkey

C. H. Goss
Division of Pulmonary and Critical Care Medicine,
University of Washington, Seattle, WA, USA

Present Address:

A. Aktas
Ataturk Education Hospital, İzmir, Turkey

Introduction

Chronic urticaria (CU), recurrent hives lasting more than 6 weeks, remains one of the diseases with unexplained pathogenetic mechanism in spite of recent developments. The term chronic spontaneous urticaria (CSU) is used to define those patients with no known cause of urticaria such as physical factors, drugs and foods. One of the proposed mechanisms in CU is autoimmunity occurring in approximately 40–50% of patients with CU [15]. There is evidence for histamine releasing activity in CU due to autoantibodies against to either IgE or high affinity IgE receptor (FcεRIα) [16]. It is suggested that these autoantibodies cause the development of wheals with mast cell activation and histamine release in dermal mast cells. These histamine releasing-autoantibodies directed against FcεRIα or IgE

can be detected in vivo by autologous serum skin test (ASST). Previous studies have shown that ASST was positive in about half of the CU patients [22, 23, 25]. However, ASST is not confined to only CU patients but it is also found in respiratory diseases such as asthma and allergic rhinitis in which the pathogenic effect of ASST is not known [9, 12, 25, 26].

Interleukin (IL)-18, previously named as IFN- γ inducing factor, has been shown to exert autoimmune regulatory activity on both Th1 and Th2 cytokines suggesting a complex role on Th1 and Th2 inflammatory responses [17, 29]. IL-18 has effects on IFN- γ , proinflammatory cytokines and other cytokines probably depending on the environment [11]. By these effects IL-18 exerts a Th1 type response requiring co-stimulation with IL-12, IL-15 or IL-2 and a Th2 type response without IL-12 and IL-23 [8]. Several studies have demonstrated that IL-18 activity is increased in Th2 type diseases such as asthma, allergic rhinitis and atopic dermatitis [2, 24, 28, 30]. IL-18 has been suggested to have a role in autoimmune activation and continuation by the effects on Th1/Th2 balance in synergy with IL-12 [8]. A previous study suggested a possible role for IL-18 which was correlated with activity of disease in ASST positive CSU patients [27].

We aimed to investigate the role of autoreactivity assessed by ASST in the patients with CSU and respiratory diseases and to investigate whether this autoreactive state is related to IL-18 levels or other clinical covariates.

Methods

Subjects

This prospective study included 55 patients with chronic urticaria (39 women and 16 men, mean age: 40.3 ± 12.3 years), 70 patients with persistent asthma (51 women and 19 men, mean age: 43.7 ± 9.6 years), 21 patients with seasonal allergic rhinitis (SAR) (14 women and 7 men, mean age: 35.5 ± 11.8 years) and 20 normal controls (12 women and 8 men, mean age: 37.7 ± 9.8).

All CSU patients were referred to our tertiary university clinic reporting recurrent, transitory, itchy weals, lasting for at least 6 weeks. Patients with physical urticaria, dermographism, vasculitis, food intolerance, allergic contact dermatitis and other skin disorders that may affect the skin test results were excluded. Activity of urticaria was assessed by determining the number and size of weals at the time when ASST was performed according to the following criteria as described by Sabroe et al. [24]: mild = 1–10 small (<3 cm in diameter), moderate = 10–50 small weals or 1–10 large weals, and severe = >50 small weals or >10 large weals.

Patients with respiratory diseases

Patients with respiratory diseases were also included in the study as a comparison group. With this aim, patients with asthma and SAR were included. Persistent asthma patients were diagnosed according to the definition of the American Thoracic Society [1]. Patients were included if they had documented asthma symptoms for at least one year. Atopy was defined by relevant medical history with positive skin prick test response to common aeroallergens. Forty asthmatics (30 women and 10 men, mean age: 45.3 ± 8.9 years) were non-atopics and 30 asthmatics (21 women and 9 men, mean age: 41.7 ± 10.3 years) were atopics according to their medical history and skin prick test results. Atopic asthmatics with sensitization to perennial allergens were included in the study. We selected newly diagnosed asthma patients without previous use of asthma medication (inhaled corticosteroids, long-acting bronchodilators, leukotriene modifiers) or previously diagnosed asthmatics that were not using asthma medication for at least 1 month before the inclusion period except short acting beta 2 agonists. Asthma severity was assessed according to GINA report as mild persistent, moderate persistent and severe persistent [20]. We did not include intermittent asthmatics in the present study.

SAR patients had documented historical data defined by nasal itching, rhinorrhea, sneezing, and nasal obstruction for at least 2 years prior to the study. The diagnosis of SAR was made on the basis of history and sensitization to pollen allergens with skin prick test (SPT). Patients with skin tests positive to mix of grasses, weeds and tree pollens correlated to the patient's seasonal history were included in the study. Allergic rhinitis patients with perennial allergen sensitization were not included. SAR patients investigated out of pollen season between November and March.

All patients and healthy controls denied a previous diagnosis of cancer, autoimmune disease or infectious disease within the previous 1 month. The study approved by the local ethics committee and all subjects gave written informed consent.

Skin prick test

Skin prick tests (SPTs) were performed by using a common panel including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, grass, tree and weed pollens, animal dander (cat, dog), animal feathers and molds (including *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*) allergen extracts (ALK, Spain). Positive and negative controls were histamine and phenolated glycerol saline, respectively. A mean wheal diameter of 3 mm or greater than that of obtained with control solution was considered as positive.

Autologous serum skin test (ASST)

Venous blood was collected in a sterile glass tubes and allowed to clot at room temperature for 30 min. Serum was separated by centrifugation at 500 g for 15 min. 50 µl autologous serum was injected intradermally into the patients forearm to perform ASST. Epidermal histamine 0.01 mg/ml and intradermal 0.9% sterile saline were used as positive and negative controls, respectively. The diameter of a wheal was calculated as the mean of the two longest perpendicular wheal diameters. ASST was considered positive when a serum-induced wheal had a diameter 3 mm greater than that of negative control surrounded by erythema at 30 min. We considered red or pink oedema as a positive response.

Serum IL-18

Blood samples were obtained from all patients and controls after an overnight fast by venipuncture. Samples were centrifuged at 2,500g for 15 min in order to separate serum and stored at -80°C until analysis. IL-18 levels in these serum samples were measured using a commercially available ELISA (Bendermed Systems, Vienna, Austria). The samples were processed according to the manufacturer's instructions. The lower limit of detection of the IL-18 assay was 9.2 pg/ml. The intra- and interassay coefficients of variation of the assay were 6.5 and 8.1%, respectively.

Study design

Clinical and demographic data were obtained using a standardized interview form. A complete physical examination was performed at the first study visit. All subjects underwent a laboratory examination including complete blood count, blood glucose, cholesterol, urea nitrogene and creatinine levels, liver and kidney function tests, thyroid function tests, antinuclear antibody, rheumatoid factor and anti-thyroid antibodies (anti-thyroglobulin, anti-TPO and anti-TSH receptor antibody). ASST and SPTs were applied to all subjects after the first examination. None of the patients was taking anti-leukotriens, immunosuppressive drugs and systemic corticosteroids within at least 1 week period before the investigation. Second generation antihistamines were stopped 5 days and first generation antihistamines were stopped 3 days before skin tests. Chronic systemic disease was defined as any other chronic organic diseases (such as diabetes, hypertension, hypercholesterolemia) other than urticaria, asthma and rhinitis and those diseases which are not mentioned in exclusion criteria.

Statistical analysis

Values are expressed as mean $\pm$ SD (standard deviation). Chi-square and Fisher's exact tests were used to compare categorical variables between groups. The Student's *t* test was used to compare ages between groups. Multiple comparisons were made between groups using Anova test and Kruskal–Wallis tests. Mann–Whitney *U* test was used to compare parameters between groups. Assay performance was computed using sensitivity and specificity. Sensitivity is the percentage of positive ASST response in the patients. Specificity is the percentage of negative ASST response in subjects without the disease.

Logistic regression analysis was used to assess the independent association between the diagnosis of CSU (reference respiratory diseases), serum IL-18 levels and the presence of ASST for all patients. The strength of the relationship between covariates and ASST was evaluated by calculating odds ratios (OR) and their 95% confidence interval (CI) for all the factors tested. Variables included in the multivariable logistic regression model were selected based on a significance test of less than 0.05 in univariate analysis. Age and IL-18 levels were analysed as continuous covariates. All other variables were coded as dichotomous categorical covariates. Differences were considered as statistically significant if $P < 0.05$. The data were analyzed with SPSS computer program for Windows version 13.0.

Results

The mean age of the patients in each group was not different from normal-controls ($P > 0.05$). Duration of disease, total IgE levels, ASST response and serum IL-18 levels in each group are presented in Table 1.

All patients with CSU and respiratory diseases

The numbers of patients who had positive response to ASST were higher in all patients when compared with normal controls (29/146 vs. 0/20, $\chi^2 = 8.24$, $P = 0.026$). Positive response to ASST in atopic and non-atopic subjects were not different (14/68 vs. 15/78, $\chi^2 = 0.04$, $P = 0.84$).

Duration of the disease was not different between patients with positive and negative response to ASST (6.7 ± 6.4 vs. 6.4 ± 5.9 years, $P = 0.81$). Fifteen patients had doctor-diagnosed hypertension and seven patients had doctor-diagnosed type II diabetes mellitus. We detected autoimmune thyroid disease in 10 patients, iron deficiency anemia in 9 patients and hypercholesterolemia in 6 patients as a result of laboratory investigation. The frequency of positive response to ASST was not different between those

Table 1 Clinical characteristics, ASST response and IL-18 levels in each group

	Chronic urticaria (<i>n</i> = 55)	Non-atopic asthma (<i>n</i> = 40)	Perennial allergic asthma (<i>n</i> = 30)	Seasonal allergic rhinitis (<i>n</i> = 21)	Normal-Controls (<i>n</i> = 20)
Duration of the disease, years	5.6 ± 8.5	7.2 ± 4.1	8.1 ± 4.4	5.8 ± 2.9	ND
Total IgE, IU/ml	215.3 ± 389.3	79.5 ± 88.6	287.7 ± 416.8	143.4 ± 205.9	ND
Atopy; yes/no	17/35	0/40	30/0	21/0	0/20
ASST, yes/no (% of positive response)	16/39 (29.1) ^a	5/35 (12.5)	5/25 (16.7)	3/18 (14.3)	0/20 (0)
Serum IL-18, pg/ml	290.0 ± 178.2 ^b	231.5 ± 228.6	240.6 ± 246.4	220.1 ± 148.5	202.2 ± 96.8

ND not determined

^a Frequency of positive response to ASST was higher in chronic urticaria than controls ($\chi^2 = 11.4$, $P = 0.004$)

^b Serum IL-18 level was higher in chronic urticaria than controls ($P = 0.031$)

patients with and without any systemic disease (12/46 vs. 17/100, $\chi^2 = 1.57$, $P = 0.27$). IL-18 levels were higher in those patients with positive ASST (301.8 ± 194.4 vs. 241.8 ± 206.3 pg/ml, $P = 0.036$). There was not statistically significant difference in regard to serum IL-18 levels between those patients with and without any systemic disease (266.0 ± 228.0 vs. 248.1 ± 193 pg/ml, $P = 0.86$) and between those patients with and without atopy (266.3 ± 223.5 vs. 243.4 ± 188.6 pg/ml, $P = 0.87$).

CSU versus respiratory diseases

Table 2 shows comparisons of atopy, thyroid autoantibodies, systemic diseases, response to ASST and serum IL-18 levels between patients with CSU and respiratory diseases. The frequency of thyroid autoimmunity (6/55 vs. 4/91, $\chi^2 = 2.19$, $P = 0.18$) and other chronic systemic diseases (22/55 vs. 24/91, $\chi^2 = 2.91$, $P = 0.10$) were similar between those patients with CSU and respiratory diseases. Atopy was increased in respiratory diseases patients ($\chi^2 = 8.87$, $P = 0.004$). Positive response to ASST ($\chi^2 = 4.6$, $P = 0.034$) and serum IL-18 levels ($P = 0.002$) were higher in CSU patients when compared with those with respiratory diseases.

Sensitivity of ASST was 29.1% for CSU and 14.3% for respiratory diseases. Specificity of the test was 100% for each of the diseases. If we take a cut-off threshold of 1.5 mm for a positive ASST response then sensitivity increases to 34.5% in CU and to 20.9% in respiratory diseases. Specificity was 90% at this threshold. If we take

6 mm cut-off threshold, then sensitivity becomes 18.2 and 11.0%, respectively in CU and respiratory diseases. The specificity was 100% at this threshold also. Sensitivity for respiratory diseases was less than that of CU at all threshold levels.

Patients with CSU

The numbers of patients who had positive response to ASST were higher in CSU patients when compared with normal controls ($\chi^2 = 11.4$, $P = 0.004$). Seventeen of CSU patients were atopic. The frequency of positive ASST response was not statistically different between atopics and non-atopics in CSU (6/17 vs. 10/38, $\chi^2 = 0.45$, $P = 0.53$). The frequency of ASST positivity was not statistically different between those patients with and without thyroid autoantibodies (3/6 vs. 13/49, $\chi^2 = 1.31$, $P = 0.34$) and any systemic disease (9/22 vs. 7/33, $\chi^2 = 2.45$, $P = 0.14$) in CSU patients. CSU patients had higher serum IL-18 levels than normal controls (Table 1) ($P = 0.031$). Serum IL-18 levels were not different in those CSU patients with or without positive ASST response (295.5 ± 203.7 vs. 276.5 ± 94.7 pg/ml, $P = 0.67$).

Twenty-seven of CSU patients were classified as mild, 21 were moderate and 7 were severe. Four of 16 CSU patients with positive response to ASST had severe disease, 8 of them moderate and 4 had mild urticaria. Positive response to ASST was determined more frequently in severe and moderate urticaria patients (42.8%) than those with mild disease (14.8%) ($P = 0.037$). Although serum

Table 2 Comparisons of atopy, thyroid autoantibodies, systemic diseases, positive response to ASST and serum IL-18 levels between patients with chronic urticaria and respiratory diseases

	Chronic urticaria (<i>n</i> = 55)	Respiratory diseases (<i>n</i> = 91)	<i>P</i>
Atopy, yes/no	17/38	51/40	0.004
Thyroid autoantibodies, yes/no	6/49	4/87	NS
Systemic diseases, yes/no	22/33	24/67	NS
Positive ASST response, yes/no	16/39	13/78	0.034
Serum IL-18, pg/ml	290.0 ± 178.2	231.9 ± 217.2	0.002

IL-18 levels were higher in severely active disease (470.9 ± 323.8) than those moderate (269.1 ± 152.4 pg/ml) and mild disease (259.2 ± 117.3 pg/ml) it did not reach statistical significance ($P = 0.062$ and 0.073 , respectively). Duration of disease was not different between those patients with positive and negative ASST response (6.2 ± 8.0 vs. 4.9 ± 8.4 years, $P = 0.87$).

Patients with respiratory diseases

Positive response to ASST was not statistically different in respiratory patients from that in controls (13/91 vs. 0/20, $\chi^2 = 5.53$, $P = 0.12$). The frequency of positive ASST response was not different between atopics and non-atopics (8/51 vs. 5/40, $\chi^2 = 0.18$, $P = 0.77$). The frequency of ASST positivity was not different between those patients with and without thyroid autoantibodies (0/4 vs. 13/87, $\chi^2 = 1.26$, $P = 1.00$) and any systemic disease (3/24 vs. 10/67, $\chi^2 = 0.09$, $P = 1.00$). Serum IL-18 levels were not different in non-atopic asthma, atopic asthma and SAR patients from normal-controls ($P > 0.05$).

Asthmatic patients

Forty-three of patients were classified as mild, 20 were moderate and 7 were severe. Four of 10 asthmatic patients with positive response to ASST were severe persistent asthmatics, 5 of were moderate persistent and 1 was mild persistent. Positive response to ASST was more frequent in severe and moderate asthma patients (33.3%) than those with mild disease (2.3%) ($P = 0.001$). Serum IL-18 levels were not different between severe (395.5 ± 284.7 pg/ml), moderate (196.7 ± 198.1 pg/ml) and mild asthmatics (227.3 ± 236.8 pg/ml) ($P > 0.05$). Duration of disease was not different between those asthmatics with positive and negative ASST response (7.6 ± 4.1 vs. 7.5 ± 4.3 years, $P = 0.99$).

Multivariate analysis for the association of ASST

Table 3 shows the multivariate logistic regression analysis in all patients. Positive response to ASST was significantly associated with diagnosis of CSU (OR: 3.13, 95% CI: 1.25–7.87) and with female gender (OR: 3.98, 95% CI: 1.19–13.38). However, serum IL-18 level was not

associated with positive ASST response (OR: 1.00, 95% CI: 0.99–1.00).

Discussion

We showed that the positive response to ASST is higher in all the patients with CU and respiratory diseases supporting the presence of the functional autoantibodies against to FcεRI or IgE. ASST reactivity was associated with disease severity in CSU and asthmatic patients. Our findings also strengthened the role of autoimmunity in CU patients having higher levels of serum IL-18. However, multivariable analysis of the present study showed that ASST response was associated mainly with diagnosis of CU and female gender.

Positive response to ASST, a condition that is associated with circulatory autoantibodies against to FcεRI or IgE, has been shown to be related with increased release of histamine in vitro [3]. Although the clinical utility is not well known, ASST is recommended to diagnose autoreactivity in CU patients. It seems to be a rare phenomenon in healthy population similar to our study [23, 26]. Our results confirmed that ASST is not sensitive enough to use as a screening test for all patients with CU, however, it has specificity as a 100%. In the present study, comparisons in “Results” for different threshold levels of ASST response suggested us that in turn there may not be difference in the pathogenesis of the ASST between urticaria and respiratory diseases. We took a wheal diameter of 3 mm larger than a negative normal saline control with surrounding erytema as in some previous studies [5–7]. Widely used 1.5 mm cut-off threshold for positive ASST response was generally adopted in order to define the statistical performance characteristics of this test with respect to in vitro serum basophil histamine release activity in urticaria patients. As stated by Konstantionu and Grattan [18] use of this 1.5 mm threshold in any other group of patients is not certain. According to our results the use of 3 mm cut-off point seems to be reasonable to compare ASST response in different subject groups. We recommend the use of this criteria to overcome a possible non-inflammatory oedema response also. Some authors have stated that false positive ASST response might occur as a result of generation of factors such as bradykinin and C5a [18]. One might think

Table 3 Association of positive response to ASST with diagnosis of chronic urticaria (CU) and IL-18 levels in multivariate logistic regression analysis

	Female gender	Having CU	Serum IL-18
Positive ASST response OR (95% CI)	3.98* (1.18–13.32)	3.13* (1.24–7.87)	1.00 (0.99–1.00)

Adjustment was made according to age and atopy, * $P < 0.05$

that chronic-systemic diseases cause false positive ASST responses through the release of some inflammatory mediators and activation of complement factors. The result of the present study showing that the patients with systemic diseases had no increased response to ASST in all as well as in CU patients did not support the possibility of false positive response.

The role of autoreactivity is not certain in the diseases. Autoantibodies against Fc ϵ RI or IgE have been implicated in the pathogenesis of asthma irrespective of atopic status [14]. Our study confirmed that the increased response to ASST in CU and respiratory diseases occurs irrespective of their atopic status. However, frequency of positive response to ASST was related with disease severity in CU and asthma suggesting autoantibodies against Fc ϵ RI or IgE might be related with disease activity.

Autoimmunity has been regarded in the pathogenesis of CU and some autoimmune diseases including thyroid autoimmune diseases that are associated with positive response to ASST [4]. We found positive thyroid autoantibody response in 10 patients including 6 CU patients. There was no statistical difference in regard to presence of anti-thyroid antibodies between those patients with CU and respiratory diseases. In addition, the presence of thyroid autoantibodies or any other chronic systemic disease might affect the occurrence of ASST response through their inflammatory actions. However, we did not find an increased ASST response in the patients with thyroid autoantibodies supporting the contention that these similar autoimmune events may mark susceptibility to autoimmunity [21]. In the present study, chronic systemic diseases alone were not associated with positive ASST response.

IL-18 may play a role in autoimmune regulatory activity [17, 29]. IL-18 is produced by monocytes/macrophages and IL-18 receptor is expressed on variety of cells including Th1 cells, naive T cells, B cells, dendritic cells, macrophages, neutrophils, NK cells, endothelial and smooth muscle cells [8]. Thus, IL-18 activity appears to exert a variety of immunological effects. It is well known that Th1 and Th17 type responses have a pathogenic role in autoimmunity [8]. IL-18 exerts a Th1 type response with co-stimulation of IL-2, IL-12 and IL-15 whereas it shows a Th17 response with co-stimulation of IL-23 [8]. IL-18 is able to generate Th2 responses under certain conditions and promote allergic inflammation through the stimulation of IL-4, IL-13 and histamine release [13]. We found that ASST was associated with serum IL-18 levels in univariate analysis of all patients suggesting a possible role of IL-18 on ASST response regardless of the underlying disease. However, we did not find any association in multivariate analysis. In addition, CSU patients had higher levels of IL-18 than normal controls. Higher IL-18 levels in CU patients

may be explained by clinical severity of the disease which could affect serum IL-18 levels as reported in a previous study [27]. Although we found higher serum IL-18 levels in severe disease in CU as well as asthma patients it did not reach statistical significance. Our multiple logistic regression analysis showed that positive response to ASST was not associated with serum IL-18 levels (OR: 1.00, 95% CI: 0.99–1.00). However, the diagnosis of CSU (OR: 3.13, 95% CI: 1.25–7.87) and female gender (OR: 3.98, 95% CI: 1.19–13.38) were associated with positive ASST response. Our results showing that female gender was found an associated risk factor for ASST reactivity confirmed results in a previous study [19]. The association of autoimmune diseases with female gender is well described; sex hormones have been implicated in this association [10]. Our results showed that women are more likely to develop autoreactivity that can be assessed by ASST.

Antihistamine use may affect the skin test response. We included only those CSU patients in whom antihistamines could stop enough time to perform skin tests to prevent false negative results. The limitation of the study is that the results do not extend to those subjects who could not stop antihistamine therapies.

In conclusion, we found that positive response to ASST is associated with CSU and female gender. IL-18, although increased in CSU patients, does not seem to be associated with positive response to ASST. CSU and asthmatic patients showed increased ASST response when their diseases are more severe. Whether this positive ASST response which is more prominent in CSU is involved in the pathogenesis of the diseases is a matter of debate. Pathogenesis of wheal response may be in part mediated by functional antibodies causing histamine release from mast cells in CU. Considering that it is also found in patients with asthma related with disease severity, it could be a result of inflammatory events such as antigen binding of specific IgE or direct vasoactive factors (thrombin and kinins) during the diseases' process.

Acknowledgments Aztra Zeneca provided a grant to support IL-18 kits and they had no role in the design of the study, analysis of the study data or writing of the manuscript. None of the authors hold a financial interest in Aztra Zeneca.

Conflict of interest None.

References

1. American Thoracic Society (1987) Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 136:225–244
2. Ando M, Shima M (2007) Serum interleukins 12 and 18 and immunoglobulin E concentrations and allergic symptoms in

- Japanese schoolchildren. *J Investig Allergol Clin Immunol* 17:14–19
3. Asero R, Lorini M, Chong SU, Zuberbier T, Tedeschi A (2004) Assessment of histamine-releasing activity of sera from patients with chronic urticaria showing positive autologous skin test on human basophils and mast cells. *Clin Exp Allergy* 34:1111–1114
 4. Asero R, Orsatti A, Tedeschi A, Lorini M (2005) Autoimmune chronic urticaria associated with type I diabetes mellitus and Graves' disease. *J Allergy Clin Immunol* 115:1088–1089
 5. Asero R, Tedeschi A, Coppola R, Griffini S, Paparella P, Riboldi P, Marzano AV, Fanoni D, Cugno M (2007) Activation of tissue factor pathway of blood coagulation in patients with chronic urticaria. *J Allergy Clin Immunol* 119:705–710
 6. Asero R, Tedeschi A, Riboldi P, Cugno M (2006) Plasma of patients with chronic urticaria shows signs of thrombin generation, and its intradermal injection causes wheal-and-flare reactions much more frequently than autologous serum. *J Allergy Clin Immunol* 117:1113–1117
 7. Bagenstose SE, Levin L, Bernstein JA (2004) The addition of zafirlukast to cetirizine improves the treatment of chronic urticaria in patients with positive autologous serum skin test results. *J Allergy Clin Immunol* 113:134–140
 8. Boraschi D, Dinarello CA (2006) IL-18 in autoimmunity: review. *Eur Cytokine Netw* 17:224–252
 9. Comi AL, Tedeschi A, Lorini M, Miadonna A (2007) Novel clinical and serological aspects in non-allergic asthma. *Res Med* 101:2526–2533
 10. Dinesh RK, Hahn BH, Singh RP (2010) PD-1, gender, and autoimmunity. *Autoimm Rev*. doi:[10.1016/j.autrev.2010.04.003](https://doi.org/10.1016/j.autrev.2010.04.003)
 11. Fantuzzi G, Dinarello CA (1999) Interleukin-18 and interleukin-1 β : two cytokine substrates for ICE (caspase-1). *J Clin Immunol* 19:1–10
 12. Guttman-Yassky E, Bergman R, Maor C, Mamorsky M, Pollack S (2007) The autologous serum skin test in a cohort of chronic urticaria patients compared to respiratory allergy patients and healthy individuals. *J Eur Acad Dermatol Venereol* 21:35–39
 13. Izakovica HL (2003) Interleukin-18 in asthma and other allergies. *Clin Exp Allergy* 33:1023–1025
 14. Jang AS, Park JS, Lee JH, Park SW, Kim DJ, Park CS (2007) Autologous serum skin test for autoantibodies is associated with airway hyperresponsiveness in patients with asthma. *Respiration* 74:293–296
 15. Kaplan A (2009) Urticaria and angioedema. In: Atkinson FN, Busse WW, Bochner BS, Holgate ST, Simons EFR, Lemanske RF (eds) *Middleton's allergy principles and practice*, 7th edn, Mosby, MO
 16. Kaplan AP (2009) Pathogenesis of chronic urticaria. *Clin Exp Allergy* 39:777–787
 17. Kohno K, Kataoka J, Ohtsuki T, Suemoto T, Okamoto I, Usui M, Ikeda M, Kurimoto M (1997) IFN- γ -inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. *J Immunol* 158:1541–1550
 18. Konstantinou GH, Grattan CEH (2009) The autologous serum skin test may be used as a marker for histamine releasing antibodies in urticaria and is not relevant to other subject groups. *Clin Exp Dermatol* 34:473–474
 19. Mari A (2004) Allergy-like asthma and rhinitis: a cross-sectional survey of a respiratory cohort and a diagnostic approach using the autologous serum skin test. *Int Arch Allergy Immunol* 133:29–39
 20. National asthma education and prevention program expert panel report 3 (2007) Guidelines for the diagnosis and management of asthma, summary report 2007. *J Allergy Clin Immunol* 120(5 Suppl 1):93–138
 21. Rottem M (2003) Chronic urticaria and autoimmune thyroid disease: is there a link? *Autoimmun Rev* 2:69–72
 22. Sabroe RA, Fiebiger E, Francis DM, Maurer D, Seed PT, Grattan CE, Black AK, Stingl G, Greaves MW, Barr RM (2002) Classification of anti-Fc ϵ RI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 110:492–499
 23. Sabroe RA, Grattan CEH, Francis DM, Barr RM, Kobza-Black A, Greaves MW (1999) The autologous serum skin test: a screening test for antibodies in chronic idiopathic urticaria. *Br J Dermatol* 140:446–452
 24. Tanaka H, Miyazaki N, Oashi K, Teramoto S, Shiratori M, Hashimoto M, Ohmichi M, Abe S (2001) IL-18 might reflect disease activity in mild and moderate asthma exacerbation. *J Allergy Clin Immunol* 107:331–336
 25. Taskapan O, Kutlu A, Karabudak O (2008) Evaluation of autologous serum skin test results in patients with chronic idiopathic urticaria, allergic/nonallergic asthma or rhinitis and healthy people. *Clin Exp Dermatol* 33:754–758
 26. Tedeschi A, Comi AL, Lorini M, Tosini C, Miadonna A (2005) Autologous serum skin test reactivity in patients with non-allergic asthma. *Clin Exp Allergy* 35:849–853
 27. Tedeschi A, Lorini M, Suli C, Asero R (2007) Serum interleukin-18 in patients with chronic ordinary urticaria: association with disease activity. *Clin Exp Dermatol* 32:568–570
 28. Verhaeghe B, Gevaert P, Holtapples G, Lukat KF, Lange B, Cauwenberge P, Bachert C (2002) Up-regulation of IL-18 in allergic rhinitis. *Allergy* 57:825
 29. Wild JS, Sigounas A, Sur N, Siddiqui MS, Alam R, Kurimoto M, Sur S (2000) IFN- γ -inducing factor (IL-18) increases allergic sensitization, serum IgE, Th2 cytokines, and airway eosinophilia in a mouse model of allergic asthma. *J Immunol* 164:2701–2710
 30. Yoshizawa X, Nomaguchi H, Izaki S, Kitamura K (2002) Serum cytokine levels in atopic dermatitis. *Clin Exp Dermatol* 27:225–229