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Potential blood biomarkers in chronic spontaneous urticaria

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Abbreviations:

AE: angioedema

ASST: autologous serum skin test

CRP: C-reactive protein

CSU: chronic spontaneous urticaria

DHEA-S: dehydroepiandrosterone sulfate

ECP: eosinophil cationic protein

FcεR: receptor of IgE Fragment c

F1+2: prothrombin fragment 1+2

FVIIa: factor VIIa

FIX: factor IX

HCS: healthy controls

IL: interleukin

MMP-9: matrix metalloproteinase-9

MPV: mean platelet volume

SP: substance P

tIgE: total immunoglobulin E

TNF: tumor necrosis factor

TPO: thyroid peroxidase

VD: vitamin D

VEGF: vascular endothelial growth factor

Abstract

Background: Chronic spontaneous urticaria (CSU) is a mast cell-driven disease that is defined as the recurrence of wheals, angioedema, or both for >6 weeks due to known or unknown causes. As of yet, disease diagnosis is purely clinical. Objective tools are needed to monitor the activity of CSU and the efficacy of treatment. Recently, several reports have suggested that blood parameters may be considered as potential disease-related biomarkers.

Objective: To review available literature on blood biomarkers for CSU diagnosis, activity monitoring, duration, patient subgroups allocation or response to treatment.

Methods: We performed a Pubmed, Google Scholar and Web of Science search and identified and analysed 151 reports published prior to January 2016.

Results: We found strong evidence for significant differences between CSU patients and healthy controls in blood levels or values of D-dimer, C-reactive protein (CRP), matrix metalloproteinase-9 (MMP-9), mean platelet volume (MPV), factor VIIa, prothrombin fragment 1+2 (F1+2), tumor necrosis factor, dehydroepiandrosterone sulfate and vitamin D. Also, there is strong evidence for a significant association between CSU activity and blood levels or values of D-dimer, F1+2, CRP, IL-6 and MPV. Strong evidence for reduced basophil count and high levels of IgG anti-FcεRI in the subgroup of CSU patients with positive autologous serum skin test was shown. In contrast, the evidence for all reported blood biomarkers for differentiating CSU from other diseases, or a role in prognosis, is weak, inconsistent or non-existent.

Conclusions & Clinical Relevance: We identified ten biomarkers which are supported by strong evidence for distinguishing CSU patients from healthy controls, or for measuring CSU

activity. There is a need for further research to identify biomarkers which predict outcome or treatment response in CSU.

Keywords: biomarkers, chronic spontaneous urticaria, disease activity, disease duration, differential diagnosis, response to treatment, subgroups

Introduction

Chronic spontaneous urticaria (CSU) is a mast cell-driven disease that is defined as the recurrence of wheals, angioedema (AE), or both for >6 weeks due to known or unknown causes [1]. At any time, 0.5-1% of the population suffer from CSU [2]. The disease has a large impact on the quality of life [3] and more than 30% of patients with moderate to severe symptoms still have CSU after 5 years [4].

Assessment of disease activity, its impact on quality of life, treatment efficacy and disease control, at present, is purely clinical and includes patient medical history, physical examination and the use of questionnaires such as the Urticaria Activity Score (UAS), the Angioedema Activity Score (AAS), the Chronic Urticaria Quality of Life (CU-Q2oL), the Angioedema Quality of Life (AE-QoL) and the Urticaria Control Test (UCT) [5]. All of these tools are validated and of great value in the monitoring of chronic urticaria patients but all have the disadvantage of being subjective, retrospective instruments. Thus, there is a lack of objective measurable indicators of the activity of the disease, and the efficacy of treatment.

Recently, several reports have suggested that blood parameters may indicate disease activity and duration and help with monitoring treatment and may be considered as potential prognostic biomarkers of CSU [6-9]. According to the National Institute of Health (NIH) Biomarkers Definitions Working Group, a biomarker is a “characteristic that is

objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention" [10].

Important characteristics of a biomarker are its specificity, sensitivity, and validity, and ideally its correlation with the activity and prognosis of the disease. The discovery, validation and implementation of a reliable biomarker could eventually lead to a personalized approach to the assessment and prediction of CSU course and the treatment of the disease and would be of great help as an objective follow-up for patients in everyday life as well as in clinical trials.

We performed a review to clarify the blood parameters that could be possible biomarkers of CSU differential diagnosis, activity, duration, patient subgroups allocation and response to treatment.

Methods

Two authors (PK and FA) independently performed a Pubmed, Google Scholar and Web of Science search of reports published prior to January 2016. They used the combination of "chronic urticaria" keyword with all of the following keywords in individual searches: "duration", "prediction", "prognosis", "treatment", "biomarker", "activity", "D-dimer", "FVII", "FXII", "F1+2", "FDP", "vitamin D", "CRP", "total IgE", "MPV", "PDW", "eosinophil count", "WBC", "basophil count", "substance P", "C3", "C4", "neopterin", "tryptase", "diamine oxidase", "CD40", "procalcitonin", "anti-TG", "anti-TPO", "eotaxin", "prolactin", "DHEA-S", "BAFF", "anti-TG", "anti-TPO", "anti-DNA", "ANA", "MMP-9", "VEGF", "TIMP-1", "CCL", "RANTES", "IL-3", "IL-4", "IL-6", "IL-9", "IL-12", "IL-17", "IL-18", "IL-23", "IL-31", "ECP", "TNF", "anti-FcεRI", and "anti-IgE".

Inclusion criteria were: 1) the studies which include CSU patients. In our analyses, we excluded cases of acute and inducible urticaria whenever possible. However, we did not exclude studies in which no distinct differentiation between CSU and chronic inducible urticaria had been made, but included this information in the tables' legends; 2) the reports in which laboratory blood parameters (protein levels in serum or plasma) including cell count were or were not significantly associated or correlated with one of the following outcomes: CSU differential diagnosis, activity, duration, patient subgroups allocation and response to treatment.

The following key exclusion criteria were used in this review: 1) non-relevant studies, e.g. studies which did not include chronic urticaria patients, where blood markers were not evaluated or an association between laboratory and clinical parameters was not analysed; 2) due to space limitations and a high number of studies available we excluded other biomarkers, or potential biomarkers, such as skin protein expression, or functional tests including basophil activation test and autologous serum skin test (ASST). These should be evaluated separately. However, we assessed the levels of potential blood biomarkers in patients with positive and negative ASST results.

We identified 2,185 studies, 2,034 of which were excluded from further analysis after review of titles, abstracts or full texts: non-relevant studies (n=1,964), reviews (n=31), case reports (n=27), and articles without abstract or full text in English (n=12). The remaining 134 studies were included in our analyses. They comprised 34 prospective studies, 11 retrospective studies, 109 case-control studies and 29 cross-sectional studies. 97 studies included only patients >16 years, 3 studies included only patients <16 years.

The rating system developed by de Croon et al. [11] was applied to each potential biomarker (Table 1). We categorized the levels of scientific evidence as "no evidence", "inconsistent",

“weak”, or “strong” based on the outcome and number of studies from different centres and teams (e.g. if one team published three studies on the same biomarker that show the same association we counted it as one study). It is important to show a consistency (reproducibility) of association between the studies, ie the association is consistent when results are replicated in studies in different settings using different methods [12]. Many studies were relevant for more than one of outcomes and were, therefore, included in more than one table. Only statistically significant findings with a P value of <0.05 were included. However, data were not adjusted for potential confounders.

Results

Potential biomarkers for confirming CSU and distinguishing it from other diseases

133 studies reported the presence or absence of significant differences in blood levels of biomarkers between CSU patients and healthy controls (HCs) or patients with other diseases (Tables 2-4, see Tables 2, 3 in the Online supplementary materials). Strong evidence for significant differences in blood levels or values of biomarkers between CSU patients and HCs was demonstrated for D-dimer (higher levels in CSU vs HCs), C-reactive protein (CRP, higher levels in CSU vs HCs), matrix metalloproteinase-9 (MMP-9, higher levels in CSU vs HCs), MPV (higher values in CSU vs HCs), factor VIIa (FVIIa, higher levels in CSU vs HCs), prothrombin fragment 1+2 (F1+2, higher levels in CSU vs HCs), tumor necrosis factor (TNF, higher levels in CSU vs HCs), dehydroepiandrosterone sulfate (DHEA-S, lower levels in CSU vs HCs) and vitamin D (VD, lower levels in CSU vs HCs). Weak evidence for a significant difference in blood levels of biomarkers between CSU patients and HCs was demonstrated for factor IX (FIX, lower levels in CSU vs HCs), complement component 4 (higher levels in CSU vs HCs),

substance P (SP, higher levels in CSU vs HCs), soluble P-selectin (higher levels in CSU vs HCs), IgE anti-thyroid peroxidase (anti-TPO, higher levels in CSU vs HCs), histamine (higher levels in CSU vs HCs), tryptase (higher levels in CSU vs HCs), vascular endothelial growth factor (VEGF, higher levels in CSU vs HCs), interleukin 4 (IL-4, higher levels in CSU vs HCs) and interleukin 17 (IL-17, higher levels in CSU vs HCs). 71 additional biomarkers were investigated, but the evidence for differences between CSU patients and HCs is inconsistent or non-existent (Table 4).

Strong evidence in blood levels of biomarkers between CSU patients and patients with other diseases was not demonstrated (Table 3, see Online supplementary materials). Weak evidence was shown for D-dimer (higher levels in CSU vs psoriasis), total IgE (lower levels in CSU vs acute urticaria) and IgG anti-FcεRI (higher levels in CSU vs atopic dermatitis).

Potential biomarkers of disease activity

75 studies reported a correlation between biomarkers and CSU activity (Tables 4, 5). Strong evidence of a significant association between blood levels or values of biomarkers and CSU activity was demonstrated only for D-dimer, CRP, F1+2, MPV and IL-6. The evidence for a significant association between CSU activity and blood levels of IL-17, MMP-9 and FVIIa was weak.

Potential biomarkers for CSU duration

15 studies reported on association of biomarker blood levels and CSU duration (Table 6), with very mixed results. Weak evidence for positive anti-thyroid antibodies and longer CSU duration was demonstrated. The evidence for other biomarkers is inconsistent or non-existent.

Potential biomarkers for CSU subgroup allocation

73 studies reported differences in blood levels of biomarkers between subgroups of CSU patients (Table 7, 8; see Table 7 in the Online supplementary materials). Strong evidence for reduced basophil count and high levels of IgG anti-FcεRI in the subgroup of CSU patients with positive autologous serum skin test was shown. Weak evidence for higher MPV and IL-17 in ASST positive CSU patients was demonstrated. The evidence for other biomarkers and presence of angioedema, age, gender, atopy, ASST and autologous plasma skin test positivity is inconsistent or non-existent.

Potential biomarkers of response to treatment

16 studies reported a correlation between levels of biomarkers and response to treatment or an association between levels of biomarkers before and after treatment (Table 9). Levels of tryptase, D-dimer and IL-31 were evaluated separately in 3 different studies during the course of omalizumab treatment. Significant association between levels of biomarkers before and after treatment was shown for D-dimer and IL-31, but not for tryptase. Levels of these parameters were elevated before treatment with consequent decrease after therapy with omalizumab. Low levels of IL-31 were associated with response to treatment. Levels of D-dimer, CRP and IL-2R, IL-5, TNF were separately evaluated in 3 studies during the course of ciclosporin treatment. Here, decreased D-dimer levels were significantly associated with response to treatment. Levels of CRP and IL-2R, IL-5, TNF were elevated before treatment with consequent decrease after therapy with ciclosporin.

There have been no studies done on biomarkers that predict response to treatment.

Thus, the level of evidence for all biomarkers is non-existent.

Correlation between potential biomarkers

43 studies reported the correlation between biomarkers (Table 10, see in the Online supplementary materials). Weak evidence for positive correlation between CRP and D-dimer and between CRP and MMP-9 was demonstrated in two and two studies from different centres, respectively. The evidence for correlation between other biomarkers is inconsistent or non-existent.

Discussion

In recent years, many publications have reported about potential biomarkers in CSU. Here, we performed a review to obtain an overview about the potential of these reported biomarkers to be used in clinical practice. The six questions asked to the literature, therefore, were: Are there potential biomarkers for the distinction of CSU from other diseases and healthy individuals? Are there potential biomarkers that correlate with CSU activity? Are there potential biomarkers that can predict CSU duration? Are there potential biomarkers that allow for subgroup allocation? Are there potential biomarkers that reflect response to treatment or predict the response? Is there a correlation between different potential biomarkers found in CSU patients?

A good biomarker should be valid in any or at least in most CSU patient populations. There is a wide variability and heterogeneity in CSU patient population from different teams, different centres and different countries (Tables 2-5). This heterogeneity of the data is the main reason why we could not conduct a meta-analysis.

The most promising results come from studies looking at D-Dimer, CRP and VD, raising the question of their possible role in the pathophysiology of urticaria. From the available literature, we found that levels of D-dimer and CRP were significantly higher in CSU patients in comparison with HCs in 9 and 8 studies from different teams, respectively. Levels of VD were significantly lower in CSU patients in comparison with HCs in 8 studies from different teams. D-dimer is a fibrin degradation product that reflects the expression of tissue factor by eosinophils, the activation of the coagulation cascade and thrombin generation. The latter can increase vascular permeability and induce degranulation of mast cells [13]. CRP is a sensitive marker that may be increased in CSU due to mast cell activation accompanied by inflammatory response [14]. VD is thought to have potential immunomodulatory activity and now has been intensely studied for its role in various chronic diseases including CSU [15].

Less convincing data exist for MMP-9, MPV, FVIIa, F1+2, TNF, IgG anti-FcεRI and DHEA-S. There have been conflicting reports on various other biomarkers such as SP, basophil count, tIgE, VEGF or IL-18 [16-21]. For example, we have described SP to be increased in CSU patients and to correlate with disease activity [22], while Tedeschi and co-workers have reported no elevation in SP in comparison to normal subjects [23].

Some studies were carried out in only 1 centre and included small samples of CSU patients that could be responsible for under- or overestimation of the results. For example, Confino-Cohen et al. retrospectively described significantly higher values of MPV in 12,778 CSU patients in comparison with 10,714 control subjects [24], whereas Isiksacan et al. found significantly decreased values of MPV but in small sample of CSU patients [25]. Importantly,

these data show that any valid biomarker may only be found by assessing large patient groups in multi-centre approaches.

Conflicting evidence might also be explained by different patient populations and differences in analysis of the results (e.g. difference in standardization between the various producers of the assay kits, the existence of various cutoffs and distinct methodological measurements of blood parameters). For instance, CRP levels were higher in CSU patients than in HCs as measured by immunoturbidimetric fully automated assay in one study [26] and were not significantly different between idiopathic CSU and HCs as measured by ELISA in another [27]. Another example would be poor agreement between manual and automated basophil counts in CSU patients [21]. In addition, the inconsistent and controversial results found in the literature might be explained, at least in part, by the treatment of patients with drugs such as corticosteroids, and the lack of exclusion of concomitant diseases that could have influence on the levels of biomarkers.

A general problem in the search for a potential biomarker is the lack of specificity of the respective marker. For example, D-Dimer or CRP levels have been shown to be elevated in CSU and correlate with disease activity. However, the problem remains as whether these substances are specific enough for urticaria, as they can often be elevated in many other diseases that are often co-morbid in CSU patients or even an underlying cause of the CSU (e.g. chronic infections, autoimmune disorders).

There are other factors that should also be taken into consideration: differences in the design of studies and different age of patients between studies, healthy controls and CSU

patients were not perfectly matched in some studies, and in some cases confounding factors could have had an influence on the outcome of potential biomarker assessment (e.g. levels of VD depend on the season and diet).

Type I and II autoimmunity are thought to be involved in the subset of CSU patients as demonstrated by the presence of IgG and IgE autoantibodies [28-30]. We evaluated the results of available studies where immunoassays (Western blot, ELISA, radioimmunoassay or immunoenzymatic assay) were used for the detection of autoantibodies. There is inconsistent and weak evidence that CSU patients have higher levels of IgG anti-FcεRI than HCs and atopic dermatitis patients, respectively.

The conflicting results of studies and the presence of autoantibodies in HCs and other diseases may be explained by different methodology, the concept of “conditional autoimmunity” and non-functional autoantibodies. For example, the direct ELISA approach used in some studies can lead to masking of IgE antibodies due to competition with IgG antibodies [30, 31]. “Conditional autoimmunity” suggests that autoantibodies can cause CSU only under certain conditions and they become pathogenic over time [32]. Fiebiger and co-workers described functional histamine-releasing IgG anti-FcεRI antibodies in CSU patients and non-functional and non-pathogenic IgG anti-FcεRI in patients with systemic lupus erythematosus, pemphigus vulgaris, dermatomyositis and bullous pemphigoid [33].

The minimum requirement for a disease biomarker is a correlation with disease activity. The most promising candidates so far are D-dimer, CRP, F1+2, IL-6 and MPV, whose levels or values were significantly higher in more severe CSU in 10, 7, 5, 4 and 3 studies from

different investigators, respectively. Elevated plasma levels of F1+2 (much like D-dimer) suggest activation of coagulation with thrombin generation [34]. MPV is a potential marker of platelet reactivity and may reflect the ongoing inflammation [24].

Inconsistent or weak evidence on other parameters such as tIgE, FVIIa, MMP-9, IL-17, IL-18, DHEA-S, SP, prolactin, VEGF, TNF and IgG anti-FcεRI may be associated with various symptom scoring systems used to assess CSU activity, sample size, age of patients, number of centres, serum or plasma used and design of study (Table 5). UAS or UAS7 were applied in 37 studies. In some studies, only patients with active urticaria or with positive ASST were recruited. Another concern was the different methodology among the studies. For example, Kessel et al. showed that high levels of total IgE were significantly associated with more severe CSU as measured by ELISA with a cutoff of 175 U/mL [17]. In contrast, Baek et al. used immunoradiometric assay with a cutoff of 91 IU/mL and found no association between total IgE and CSU severity [35].

There is weak or conflicting evidence on the association between levels of anti-thyroid antibodies, VD, tIgE and disease duration (Table 6). Such controversy may be, in part, explained by differences in patient selection, samples size and study design as well as in the duration of the follow-up period. For example, Woo et al. did not analyse factors that affect the levels of serum VD (e.g. smoking, daily sunlight per subject, baseline nutritional status, time of blood sampling) due to retrospective design of study [15]. Moreover, other investigations of VD were limited by small sample size, selection and detection bias [36-38] and in some studies no specific criteria for urticaria remission were described [4, 37, 39]. For

other parameters such as IgG or IgE autoantibodies no significant association was found.

Taken together, there is currently no known predictor of disease duration and further studies are required to identify whether such predictors do exist.

Up to 40-50% of CSU patients have autoreactive urticaria as defined by positive ASST [40].

We found strong evidence for a high prevalence of IgG anti-FcεRI in ASST positive CSU patients [41-43]. Three studies from different centres and teams provided evidence that positive ASST is linked to reduced basophil numbers [44-46]. Evidence for basopenia in autoimmune CSU is consistent with the hypothesis that circulating basophils may be recruited from blood into urticarial wheals during disease activity [21]. Significant associations between ASST positivity and levels of potential biomarkers were shown in some studies for MPV, IL-17, ATA, CRP, IL-18, ANA but not in other. IgE anti-TPO and anti-dsDNA were not associated with autoreactivity. Thus, positive ASST may be linked only to IgG autoantibodies or other histamine-releasing factors.

It is as of yet unclear why some patients with CSU present with wheals only while others have wheals and angioedema or angioedema only. Therefore, it would be of great interest to identify biomarker associated with the absence or presence of angioedema in CSU.

However, no such biomarker has been identified yet and the presence of angioedema was shown to be not associated with altered levels of anti-FcεRI, anti-IgE, SP, B-cell activation factor and tryptase.

Therefore, future investigations are needed to reliably identify CSU subgroups.

The emergence of new therapies in the treatment of urticaria emphasizes the demand of a biomarker in order to objectively assess the response to therapy or to predict the outcome of a therapy. The constant need for new therapies aims at the maximum efficacy and the best fit for individual patients. Studies fall short on measuring the response to treatment, forgetting often to correlate changes in substances and UAS at different time points. Significant association between levels of biomarkers before and after treatment with omalizumab, ciclosporin or vitamin D supplementation was shown for D-dimer, IL-31 (omalizumab), CRP, IL-2R, IL-5, TNF (ciclosporin) and VD, respectively. Significant association was shown between levels of D-dimer and IL-31 and response to treatment with ciclosporin and omalizumab, respectively. These two biomarkers may be the most promising candidates, but further research is needed to verify this and or to identify other potential biomarkers.

A correlation between biomarkers may be important in understanding of pathogenetic processes in CSU and evaluation of two or more biomarkers in the same patient may increase a prognostic value. Non-conflicting weak evidence was only shown for a positive correlation between the levels of D-dimer and CRP as well as CRP and MMP-9. The elevation of D-dimer and CRP in CSU and correlation between them and with disease activity may support the involvement and the close link between coagulation activation and inflammation in CSU pathogenesis [34].

Conclusion

We identified ten biomarkers which are supported by strong evidence for distinguishing CSU patients from healthy controls, or for measuring CSU activity. There is a need for further research to identify biomarkers which predict outcome or treatment response in CSU.

Table 1. Definitions of levels of evidence [11]

Levels of evidence for “association”	Description
Strong	3 studies available that find an association in the same direction or ≥ 4 studies available, of which $>66\%$ find a significant association in the same direction and no more than 25% find an opposite association
Weak	2 studies available that find a significant association in the same direction or 3 studies available, of which 2 find a significant association in the same direction and the third study finds no significant association
No evidence	≤ 1 study available
Inconsistent	Remaining cases
Levels of evidence for “no association”	Description
Strong	>4 studies are available, of which $>85\%$ find no association
Weak	>4 studies are available, of which $>75\%$ find no association

Table 4. Evidence on potential biomarkers as tools for differential diagnosis and evaluating of CSU activity

Group	Blood biomarkers		n of studies with statistically significant association in levels or values of potential biomarkers between CSU and healthy controls						n of studies with statistically significant association between levels or values of potential biomarkers and CSU activity							
	Levels or values	Potential biomarker	Shown			Not shown			Overall evidence ¹	Shown			Not shown			Overall evidence ¹
			Total	From different centres	Sample ≥50 CSU patients	Total	From different centres	Sample ≥50 CSU patients		Total	From different centres	Sample ≥50 CSU patients	Total	From different centres	Sample ≥50 CSU patients	
Coagulation and inflammation	↑	D-dimer	13	9	3	0	0	0	Strong	12	10	4	1	1	0	Strong
	↑	TAT	2	1	1	0	0	0	No	1	1	1	0	0	0	No
	↑	Fibrinogen	1	1	0	2	2	1	Inconsistent	0	0	0	0	0	0	No
	↑	F1+2	8	3	2	1	1	0	Strong	8	5	2	0	0	0	Strong
	↑	FVIIa	4	3	1	1	1	1	Strong	3	2	1	1	1	0	Weak
	↓	FXIIa, FXII	1	1	1	2	2	1	Inconsistent	0	0	0	0	0	0	No
	↑	FDP	1	1	0	0	0	0	No	1	1	1	1	1	0	No
	↓	FVIII	1	1	1	0	0	0	No	0	0	0	0	0	0	No
	↓	FIX	2	2	2	0	0	0	Weak	0	0	0	0	0	0	No
	↓	FXI	1	1	1	0	0	0	No	0	0	0	0	0	0	No
	↓	FII	1	1	1	0	0	0	No	0	0	0	0	0	0	No
	↑	aPTT	1	1	1	2	2	0	Inconsistent	0	0	0	0	0	0	No
	↑	PT	0	0	0	3	3	1	Inconsistent	0	0	0	0	0	0	No
	↓	TF	1	1	1	0	0	0	No	0	0	0	0	0	0	No
	↓	TM	1	1	1	0	0	0	No	0	0	0	0	0	0	No
↑	CRP	13	8	7	3	2	1	Strong	10	7	6	3	3	0	Strong ³	
↑	ESR	1	1	0	0	0	0	No	0	0	0	0	0	0	No	
Enzymes, vitamins	↓	Vitamin D	8	8	5	0	0	0	Strong	2	2	1	6	6	3	No ²
	↓	B ₁₂	1	1	0	0	0	0	No	0	0	0	0	0	0	No
	↑	MMP-9	3	3	2	0	0	0	Strong	2	2	1	1	1	1	Weak
	↑	TIMP-1	1	1	1	0	0	0	No	0	0	0	0	0	0	No
	↑	MIP-1α	1	1	0	1	1	1	Inconsistent	0	0	0	0	0	0	No
	↓	DAO	0	0	0	1	1	1	No	0	0	0	0	0	0	No
Hormones	↑	Prolactin	1	1	1	2	2	0	Inconsistent	1	1	1	2	2	0	Inconsistent
	↑	PCT	1	1	0	0	0	0	No	0	0	0	0	0	0	No
	↓	DHEA-S	7	3	2	0	0	0	Strong	1	1	0	3	3	1	Inconsistent
	↑/↓	Cortisol	0	0	0	1	1	0	No	0	0	0	1	1	0	No
Complement	↑	C4	2	2	2	0	0	0	Weak	1	1	1	0	0	0	No
	↑	C3	1	1	1	1	1	1	Inconsistent	1	1	1	0	0	0	No
	↑	CSa	1	1	1	1	1	1	Inconsistent	0	0	0	1	1	1	No
Neuropeptides	↑	SP	2	2	2	1	1	1	Weak	1	1	1	1	1	1	Inconsistent

	↑	SCF	1	1	1	0	0	0	No	0	0	0	1	1	1	No
	↑	NPY, NGF, VIP, CGRP	0	0	0	1	1	1	No	0	0	0	1	1	1	No
Cells, receptors	↑	sCD40	1	1	1	0	0	0	No	0	0	0	1	1	1	No
	↑	sCD40L	1	1	1	1	1	1	Inconsistent	0	0	0	1	1	1	No
	↑	sCD154	1	1	0	0	0	0	No	0	0	0	0	0	0	No
	↑	Platelet count	1	1	0	7	5	4	No ²	1	1	1	2	1	1	Inconsistent
	↑	WBC	1	1	1	2	2	0	Inconsistent	0	0	0	0	0	0	No
	↑	MPV	4	4	3	3	2	1	Strong ³	3	3	2	0	0	0	Strong
	↓	Basophil count	6	4	1	2	2	1	Inconsistent	1	1	0	0	0	0	No
	↑	Eosinophil count	2	2	1	4	4	3	Inconsistent	1	1	0	0	0	0	No
	↑	PDW	1	1	0	2	2	1	Inconsistent	0	0	0	0	0	0	No
	↑	sPS	2	2	0	1	1	0	Weak	1	1	0	1	1	0	Inconsistent
	↓	Hemoglobin	0	0	0	2	2	1	Inconsistent	0	0	0	0	0	0	No
Autoantibodies, immunoglobulins	↑	IgG anti-FcεRI	8	4	5	3	2	2	Inconsistent	2	1	2	1	1	1	Inconsistent
	↑	IgG anti-IgE	4	3	3	4	3	1	Inconsistent	2	1	2	1	1	1	Inconsistent
	↑	IgE anti-dsDNA	1	1	1	0	0	0	No	0	0	0	0	0	0	No
	↑	IgG anti-dsDNA	0	0	0	2	2	0	Inconsistent	0	0	0	0	0	0	No
	↑	IgE anti-TPO	2	2	2	1	1	0	Weak	0	0	0	2	2	2	Inconsistent
	↑	ANA	1	1	1	2	2	1	Inconsistent	0	0	0	1	1	1	No
	↑	ATA	16	16	12	6	6	3	Inconsistent	2	2	2	3	3	2	Inconsistent
	↑	APC	1	1	1	1	1	0	Inconsistent	0	0	0	0	0	0	No
	↑	ATG	0	0	0	1	1	1	No	0	0	0	0	0	0	No
	↑	ACL	0	0	0	1	1	1	No	0	0	0	0	0	0	No
	↑	Total IgE	5	4	3	5	5	5	Inconsistent	1	1	1	2	2	0	Inconsistent
Mediators	↑	Histamine	2	2	1	1	1	1	Weak	0	0	0	2	2	2	Inconsistent
	↑	Tryptase	2	2	2	0	0	0	Weak	1	1	1	0	0	0	No
	↑	ECP	1	1	0	1	1	1	Inconsistent	1	1	0	0	0	0	No
	↑	VEGF	2	2	2	1	1	1	Weak	1	1	1	1	1	1	Inconsistent
	↑	ES, TSP-1	1	1	1	0	0	0	No	0	0	0	1	1	1	No
	↑	sICAM-1, sVCAM-1	1	1	1	1	1	0	Inconsistent	0	0	0	0	0	0	No
	↑	ELAM-1	1	1	0	0	0	0	No	0	0	0	0	0	0	No
Cytokines	↑	IL-2R, IL-2	2	1	1	1	1	0	Inconsistent	0	0	0	0	0	0	No
	↑	IL-4	2	2	1	2	1	1	Weak	0	0	0	0	0	0	No
	↑	IL-5	1	1	0	0	0	0	No	0	0	0	0	0	0	No
	↑	IL-6	5	3	2	2	2	2	Inconsistent	4	4	3	1	1	1	Strong
	↑	IL-9	0	0	0	1	1	1	No	0	0	0	1	1	1	No
	↑	IL-10	1	1	0	1	1	0	Inconsistent	0	0	0	0	0	0	No
	↑	IL-13	1	1	1	0	0	0	No	0	0	0	0	0	0	No
	↑	IL-17	2	2	1	1	1	0	Weak	2	2	1	0	0	0	Weak
	↑	IL-18	2	2	2	3	3	2	Inconsistent	2	2	1	3	3	3	Inconsistent
	↑	IL-23	1	1	1	0	0	0	No	1	1	1	0	0	0	No

	↑	IL-31	1	1	0	1	1	1	Inconsistent	0	0	0	3	1	1	No
	↑	IL-33	0	0	0	2	2	2	Inconsistent	0	0	0	2	2	2	Inconsistent
	↑	TNF	3	3	1	1	1	1	Strong	1	1	1	1	1	1	Inconsistent
	↑	IFN γ	0	0	0	3	2	2	Inconsistent	0	0	0	0	0	0	No
	↑	TGF- β 1	1	1	0	1	1	0	Inconsistent	1	1	0	0	0	0	No
	↑	BAFF	1	1	0	0	0	0	No	1	1	0	0	0	0	No
Chemokines	↑	TSLP	0	0	0	1	1	1	No	0	0	0	1	1	0	No
	↑	Eotaxin	1	1	1	1	1	1	Inconsistent	0	0	0	1	1	1	No
	↑	CXCL8	1	1	0	1	1	1	Inconsistent	0	0	0	0	0	0	No
	↑	CXCL9	1	1	0	0	0	0	No	0	0	0	0	0	0	No
	↑	CXCL10	1	1	0	1	1	1	Inconsistent	0	0	0	0	0	0	No
	↑	CCL27	0	0	0	1	1	0	No	1	1	0	0	0	0	No
	↑	CCL11	1	1	0	0	0	0	No	0	0	0	1	1	0	No
	↑	CCL26	1	1	0	0	0	0	No	1	1	0	0	0	0	No
	↑	CCL17	1	1	0	0	0	0	No	1	1	0	0	0	0	No
Other	↑	CCL5/RANTES	1	1	1	1	1	0	Inconsistent	0	0	0	0	0	0	No
	↑	CCL2	1	1	0	0	0	0	No	0	0	0	0	0	0	No
	↑	MGP	0	0	0	1	1	0	No	0	0	0	1	1	0	No
	↑	PTX3	0	0	0	1	1	0	No	1	1	0	0	0	0	No
	↓	pSOD	1	1	0	0	0	0	No	0	0	0	1	1	0	No
	↓	pGPx	1	1	0	0	0	0	No	1	1	0	0	0	0	No
	↑	pMDA	1	1	0	0	0	0	No	1	1	0	0	0	0	No
	↑	NPT	1	1	0	1	1	1	Inconsistent	0	0	0	1	1	0	No

DD – D-dimer; F – factor; F1+2 – prothrombin fragment 1+2; TPO – thyroid peroxidase; CCL – chemokine (C-C motif) ligand; aPTT – activated partial thromboplastin time; WBC – white blood cells; ETP – endogenous thrombin potential; PT – prothrombin time; MPV – mean platelet volume; PDW – platelet distribution width; CRP – C-reactive protein; VEGF – vascular endothelial growth factor; IL – interleukin; MGP – matrix GLA protein; PTX3 – pentraxin 3; ECP – eosinophil cationic protein; sPS – soluble P-selectin; ES – endostatin; TPS-1 – thrombospondin-1; SP – substance P; TGF- β – transforming growth factor- β ; ESR – erythrocyte sedimentation rate; DHEA-S – dehydroepiandrosterone sulfate; SCF – stem cell factor; NPY – neuropeptide Y; NGF – nerve growth factor; VIP – vasoactive intestinal peptide; CGRP – calcitonin gene-related peptide; TNF – tumor necrosis factor; ATA – anti-thyroid antibodies; TSLP – thymic stromal lymphopoietin; pSOD – platelet superoxide dismutase; pGPx – platelet glutathione peroxidase; pMDA – platelet malondialdehyde; dsDNA – double stranded DNA; TAT – thrombin-antithrombin complex; C5a, C3, C4 – complement component 5a, 3, 4; TF – tissue factor; TM – thrombomodulin; DAO – diamine oxidase; sICAM-1, sVCAM-1 – soluble adhesion molecules; CXCL – chemokine (C-X-C motif) ligand; PCT – procalcitonin; sCD40/sCD40L – soluble CD40/CD40L; ANA – antinuclear antibodies; BAFF – B-cell activation factor; NPT – neopterin; FDP – fibrin degradation products; MMP-9 – matrix metalloproteinase-9; TIMP-1 – tissue inhibitor of metalloproteinase-1; ELAM-1 – endothelial-leukocyte adhesion molecule 1; IFN γ – interferon gamma; MIP-1 α – macrophage inflammatory protein 1 α ; sCD154 – soluble CD154; ATG – anti-transglutaminase IgA antibodies; ACL – anti-cardiolipin antibodies; APC – anti-parietal cell antibodies; ¹based on statistically significant findings with a P value of <0.05 and the levels of evidence listed in Table 1 (i.e. on number of studies from different teams and centres); ²weak evidence for “no association”; ³overall evidence was calculated taking into account a high number of studies with sample >50 CSU patients

Table 5. Are there potential biomarkers that correlate with CSU activity?

Authors	Year	n, CSU	Significant association between levels or values of potential biomarkers and CSU activity		Assessment of activity	Blood	Age, y	Design	Country	Ref
			Yes*	No						
Wang et al.	2015	103	↑ DD, FVIIa, F1+2	–	–	P	–	CS	China	[47]
Shin et al.	2015	96	–	IgE anti-TPO	UAS	S	>16	CS	Korea	[31]
Lu et al.	2015	24	↑ DD, CCL17, CCL26, CCL27	CCL11, tIgE, CRP	UAS	S	>16	P, CC	China	[48]
Cugno et al.	2015	30 ¹	↑ F1+2, DD ¹ ; ↓ F1+2, DD ²	–	Other	P	>16	CC	Italy	[34]
Aleem et al.	2015	194 ⁵	↑ MPV	Platelet count	TUSS	–	<16>	P, CC	India	[49]
Jagodzinska et al.	2015	82 ¹	–	VEGF	UAS	P	>16	CS	Poland	[18]
Grzanka et al.	2015	17	–	MGP	UAS	S	>16	CC	Poland	[9]
Woo et al.	2015	72	↓ Vitamin D	–	UAS	S	<16>	R, CC	Korea	[15]
Movahedi et al.	2015	114	↑ Vitamin D	–	UAS	S	>16	CC	Iran	[50]
Kasperska-Zajac et al.	2015	42	↑ PTX3, CRP	–	UAS	P, S	>16	CC	Poland	[26]
Oguz Topal et al.	2015	58	–	Vitamin D	UAS	S	<16>	P, CC	Turkey	[51]
Farres et al.	2015	30 ¹	↑ DD ¹ ; ↓ DD, FVIIa ^{2,3}	FVIIa ³	Other	P	>16	CC	Egypt	[52]
Lunge et al.	2015	50	↑ ATA	–	–	S	>16	CS	India	[53]
Palikhe et al.	2014	43 ⁵	–	Soluble P-selectin	UAS	P	>16	CC	Korea	[54]
Kasperska-Zajac et al.	2014	66	↑ Platelet count	–	UAS	S	>16	CS	Poland	[55]
Chandrashekar et al.	2014	45	↑ PA, soluble P-selectin, MPV	–	USS	–	>16	CS	India	[56]
Baek et al.	2014	48	↑ DD	CRP, tIgE	UAS	–	>16	R	Korea	[35]
Puxeddu et al.	2014	106	–	ES, TSP-1	UAS7	S	>16	CC	Italy	[57]
Rasool et al.	2014	62	↑ IL-18, IL-6	–	Other	S	<16>	CC	India	[58]
Metz et al.	2014	118	↑ Substance P	–	UAS7	S	–	CC	Germany	[22]
Abdel-Rehim et al.	2014	22	–	Vitamin D	UAS	S	>16	CC	Egypt	[36]
Chandrashekar et al.	2014	45	↓ Vitamin D, ↑ IL-17, TGF-β1	–	USS	S	>16	CS	India	[59]
Ucmak et al.	2014	48	–	DHEA-S, Pr	UAS	P, S	>16	CC	Turkey	[60]
Basak et al.	2014	57 ⁵	–	Substance P, SCF, NGF, CGRP, VIP, NPY	UAS	S	>16	CC	Turkey	[61]
Grzanka et al.	2014	35	↑ CRP	Vitamin D	UAS	S	>16	CC	Poland	[37]
Boonpiyathad et al.	2014	60	–	Vitamin D	UAS7	S	>16	P, CC	Thailand	[62]
Altrichter et al.	2014	39	–	IL-31	UAS7	S	–	P	Germany	[63]
Atwa et al.	2014	75	↑ IL-17, IL-23, TNF	–	UAS7	S	<16>	CC	South Arabia	[64]
Aleem et al.	2014	194 ⁵	↑ CRP	–	Other	–	<16>	CC	India	[65]
Triwongwanarat et al.	2013	120	↑ DD	–	UAS	P	>16	R	Thailand	[66]
Metz et al.	2013	>62	–	Hs, TNF, C5a, IL-6, 9, 18, 31, 33, TSLP	UAS7	S, P	>16	CC	Germany	[16]

Criado et al.	2013	22 ⁵	↑DD ¹ , ↓DD ²	–	–	S	>16	CC	Brazil	[67]
Rajappa et al.	2013	45	↑pMDA, pSOD, pGPx, IL-6, CRP	Platelet count	USS	–	>16	CS	India	[68]
Zhu et al.	2013	130	↑F1+2, TAT, DD, HMWK	–	UAS	P, S	>16	P, CC	China	[69]
Cho et al.	2013	75	–	Hs	SSS	P	>16	CC	Korea	[70]
Ucmak et al.	2013	53	↑IL-6, CRP	–	UAS	S, P	>16	CC	Turkey	[71]
Puxeddu et al.	2013	73	–	IL-33, sST2, IL-18, IL-18BP	UAS7	S	<16>	CC	Italy	[19]
Kasperska-Zajac et al.	2013	70 ¹	↑C3, C4, CRP	–	UAS	S	–	CC	Poland	[72]
Kasperska-Zajac et al.	2013	21 ¹	↑PCT	–	UAS	S	>16	CC	Poland	[73]
Jasinska et al.	2013	67	–	sCD40	UAS	P	>16	CS	Poland	[74]
Kamel-Sabry et al.	2013	60	↑Pr	DHEA-S	Other	S	>16	CC	Egypt	[75]
Jasinska et al.	2012	67	–	sCD-40L	UAS	P	>16	CS	Poland	[76]
Kessel et al.	2012	46	↑BAFF	–	–	S	>16	P, CC	Israel	[77]
Tedeschi et al.	2012	100	↑CRP ¹ , ↓CRP ²	Eotaxin	UAS	S	>16	CC	Italy	[78]
Magen et al.	2012	749	–	ATA	UAS	S	>16	R	Israel	[79]
Kurt et al.	2011	55	–	IL-18	Other	S	>16	P, CC	Turkey	[80]
Altrichter et al.	2011	478	–	IgE anti-TPO	UAS	S	>16	CC	Germany	[29]
Kasperska-Zajac et al.	2011	58	↑IL-6, CRP ¹ , ↓IL-6, CRP ²	–	UAS	P, S	>16	CC	Poland	[14]
Ciprandi et al.	2011	28	–	NPT	UAS	S	<16>	CC	Italy	[81]
Asero et al.	2011	43	↑F1+2, DD	CRP	UAS	P	>16	CC	Italy	[27]
Takeda et al.	2011	36	↑DD, F1+2, SFMC	FDP	Other	P	<16>	CS	Japan	[82]
Magen et al.	2010	289	↑MPV	–	CUSS	–	>16	R, CC	Israel	[45]
Takahagi et al.	2010	82	↑FDP, DD, CRP ¹	–	Other	P, S	<16>	R	Japan	[83]
Raap et al.	2010	46	–	IL-31	Other	S	>16	CS	Germany	[84]
Thorp et al.	2010	25 ⁵	–	Vitamin D	–	–	>16	CC	USA	[38]
Tedeschi et al.	2010	52	↑MMP-9, CRP ¹ ; ↓MMP-9, CRP ²	–	USS	P	>16	CC	Italy	[85]
Kessel et al.	2010	203	↑tIgE, ATA	–	Other	S	>16	P, CC	Israel	[17]
Ferrer et al.	2010	81	↑Tryptase	–	Other	S	>16	CC	Spain	[86]
Wang et al.	2009	30	↑FVIIa	–	Other	–	>16	CC	China	[87]
Tedeschi et al.	2009	80	↑VEGF	–	–	P	>16	CC	Italy	[88]
Altrichter et al.	2009	70	–	MMP-9	UAS	P	>16	CC	Germany	[8]
Asero et al.	2008	21	↑DD, F1+2 ¹ ; ↓DD, F1+2 ²	–	Other	P	>16	CS	Italy	[89]
Kasperska-Zajac et al.	2008	32	↓DHEA-S ¹	–	–	S	>16	CC	Poland	[90]
Eckman et al.	2008	73	–	Anti-FcεRI, anti-IgE	Other ^{1,2}	S	–	CC	USA	[91]
Asero et al.	2007	37	↑F1+2	DD	Other	P	<16>	CC	Italy	[92]
Brzoza et al.	2007	33	–	DHEA-S, cortisol, Pr	–	S	>16	CS	Poland	[93]
Tedeschi et al.	2007	34	↑IL-18	–	Other	S	>16	CS	Italy	[94]
Asero et al.	2006	28	↑F1+2	–	Other	P	>16	CC	Italy	[95]
Kessel et al.	2005	29	↑MMP-9	–	Other	P	>16	CC	Israel	[96]
Verneuil et al.	2004	45 ⁵	–	ATA	–	S	>16	P, CC	France	[97]
Toubi et al.	2004	139	–	ANA, ATA	Other	–	>16	P, CC	Israel	[4]
Grattan et al.	2003	7	Basophil count	–	UAS	–	>16	CC	UK	[21]

Sabroe et al.	2002	78	↑Anti-FcεRI, anti-IgE ⁴	–	USS	S	<16>	CC	UK, Austria	[42]
Sabroe et al.	1999	107	↑Anti-FcεRI, anti-IgE ⁴	–	USS	S	<16>	P	UK, Austria	[98]
Lorenzo et al.	1996	25	↑Eosinophil count, ECP ¹	–	Other	S	–	CC	Italy	[99]

*↑ or ↓ – patients with more severe disease had higher or lower levels or values of markers; – not defined or no data; P – plasma; S – serum; DD – D-dimer; F – factor; F1+2 – prothrombin fragment 1+2; TPO – thyroid peroxidase; CCL – chemokine (C-C motif) ligand; CRP – C-reactive protein; tIgE – total IgE; MPV – mean platelet volume; VEGF – vascular endothelial growth factor; MGP – matrix GLA protein; PTX3 – pentraxin 3; PA – platelet aggregation; ES – endostatin; TPS-1 – thrombospondin-1; IL – interleukin; TGF-β1 – transforming growth factor-β; DHEA-S – dehydroepiandrosterone sulfate; Pr – prolactin; SCF – stem cell factor; NPY – neuropeptide Y; NGF – nerve growth factor; VIP – vasoactive intestinal peptide; CGRP – calcitonin gene-related peptide; TNF – tumor necrosis factor; Hs – histamine; TSLP – thymic stromal lymphopoietin; C5a, C3, C4 – complement component 5a, 3, 4; pSOD – platelet superoxide dismutase; pGPx – platelet glutathione peroxidase; pMDA – platelet malondialdehyde; TAT – thrombin-antithrombin complex; HMWK – high molecular weight kininogen; PCT – procalcitonin; sCD40/sCD40L – soluble CD40/CD40L; BAFF – B-cell activation factor; FDP – fibrin degradation products; SFMC – soluble fibrin monomer complex; MMP-9 – matrix metalloproteinase-9; ATA – anti-thyroid antibodies; ANA – antinuclear antibodies; ECP – eosinophil cationic protein; UAS – urticaria activity score; TUSS – total urticaria severity score; USS – urticaria severity score; CUSS – chronic urticaria severity score; SSS – scale of symptom severity; R – retrospective study; P – prospective study; CS – cross-sectional study; CC – case-control study; ¹active CSU; ²during remission; ³factor VIIa levels did not differ significantly according to disease severity grades but after complete disease remission, there was a significant dramatic drop in levels of FVIIa among patients; ⁴patients with serum histamine-releasing activity had more severe urticaria than patients without such activity; ⁵chronic inducible urticaria was not excluded or it is unclear from the paper;

Table 6. Are there potential biomarkers that can predict CSU duration?

Authors	Year	n, CSU	Parameters	Significant association ¹	Blood	Age, y	Design	Country	Ref
Woo et al.	2015	72	Vitamin D	Yes	S	<16>	R, CC	Korea	[15]
Kasperska-Zajac et al.	2014	66	MPV, PDW, PC, CRP	No	S	>16	CS	Poland	[55]
Metz et al.	2014	118	Substance P	No	S	–	CC	Germany	[22]
Abdel-Rehim et al.	2014	22	Vitamin D	No	S	>16	CC	Egypt	[36]
Grzanka et al.	2014	35	Vitamin D	No	S	>16	CC	Poland	[37]
Hatada et al.	2013	85	IgE anti-dsDNA	No	S	>16	CC	Japan	[30]
Siles et al.	2013	205	Tryptase	No	S	>16	R	USA	[39]
Altrichter et al.	2011	478	IgE anti-TPO	No	S	>16	CC	Germany	[29]
Thorp et al.	2010	25 ²	Vitamin D	No	–	>16	CC	USA	[38]
Kessel et al.	2010	203	Total IgE	Yes	S	>16	P, CC	Israel	[17]
Nebiolo et al.	2009	228	ATA, ANA	No	S	>16	P	Italy	[100]
Gangemi et al.	2009	95	ATA	Yes	S	>16	R, CC	Italy	[101]
Altrichter et al.	2009	70	MMP-9	No	P	>16	CC	Germany	[8]
Toubi et al.	2004	139	ATA, total IgE, ANA	Yes (ATA), No (tIgE, ANA)	–	>16	P, CC	Israel	[4]
Sabroe et al.	1999	107	Anti-FcεRI, anti-IgE	No	S	<16>	P	UK	[98]

– not defined or no data; P – plasma; S – serum; MPV – mean platelet volume; PDW – platelet distribution width; PC – platelet count; CRP – C-reactive protein; dsDNA – double stranded DNA; TPO – thyroid peroxidase; ATA – anti-thyroid antibodies; MMP-9 – matrix metalloproteinase-9; ANA – antinuclear antibodies; R – retrospective study; P – prospective study; CC – case-control study; ¹between levels or values of potential biomarkers and longer CSU duration; ²chronic inducible urticaria was not excluded or it is unclear from the paper

Table 8. Evidence on potential biomarkers in ASST positive CSU patients

Blood biomarkers		n of studies where levels or values of potential biomarkers are statistically significantly higher or lower in ASST+ CSU patients compared to ASST- CSU patients						
Levels or values	Potential biomarker	Association is shown			Association is not shown			Overall evidence ¹
		Total	From different centres	Sample >100 CSU patients	Total	From different centres	Sample >100 CSU patients	
↓	Basophil count	3	3	1	0	0	0	Strong
↑	IgG anti-FcεRI	3	3	1	1	1	0	Strong
↑	MPV	2	2	2	0	0	0	Weak
↑	IL-17	2	2	0	1	1	0	Weak
↑	D-dimer	0	0	0	2	2	1	Inconsistent
↑	CRP	2	2	2	6	5	0	Inconsistent
↑	Platelet count	0	0	0	2	2	2	Inconsistent
↓	Vitamin D	2	2	0	2	2	0	Inconsistent
↑	ATA	2	2	0	5	5	1	Inconsistent
↑	TNF	1	1	0	1	1	0	Inconsistent
↑	IgG anti-IgE	1	1	0	3	3	1	Inconsistent
↑	IL-18	2	2	0	3	3	0	Inconsistent
↑	sCD-40/L	1	1	0	2	2	0	Inconsistent
↑	Prolactin	1	1	0	1	1	0	Inconsistent
↑	ANA	1	1	1	2	2	0	Inconsistent
↑	C3, C4	0	0	0	2	2	0	Inconsistent
↓	DHEA-S	0	0	0	3	3	0	Inconsistent
↑	Eotaxin	0	0	0	2	2	1	Inconsistent
↑	MMP-9	0	0	0	2	2	0	Inconsistent
↑	sVCAM-1	0	0	0	2	2	0	Inconsistent
↑	Total IgE	1	1	1	4	4	2	No ²
↑	TGF-β	1	1	0	0	0	0	No
↑	IL-23	1	1	0	0	0	0	No
↑	F1+2	1	1	0	0	0	0	No
↑	IL-13	1	1	0	0	0	0	No
↑	Tryptase	1	1	0	0	0	0	No
↑	IL-6	0	0	0	3	3	0	No
↑	ES, TSP-1	0	0	0	1	1	1	No
↑	PTX3	0	0	0	1	1	0	No
↑	IgE anti-dsDNA	0	0	0	1	1	0	No
↑	IgE anti-TPO	0	0	0	1	1	1	No
↑	BAFF	0	0	0	1	1	0	No
↑	IL-10	0	0	0	1	1	0	No

¹based on statistically significant findings with a P value of <0.05 and the levels of evidence listed in Table 1 (i.e. on number of studies from different teams and centres); ²weak evidence for “no association”; MPV – mean platelet volume; IL – interleukin; CRP – C-reactive protein; ATA – anti-thyroid antibodies; TNF – tumor necrosis factor; ANA – antinuclear antibodies; C3, C4 – complement component 3, 4; DHEA-S – dehydroepiandrosterone sulfate; MMP-9 – matrix metalloproteinase-9; sVCAM-1 – soluble adhesion molecules; TGF-β – transforming growth factor-β; F1+2 – prothrombin fragment; ES – endostatin; TPS-1 – thrombospondin-1; PTX3 – pentraxin 3; dsDNA – double stranded DNA; BAFF – B-cell activation factor; TPO – thyroid peroxidase

Table 9. Are there potential biomarkers that reflect response to treatment or predict the response?

Authors	Year	n, CSU	n, healthy controls	Drug(s)	Dose	Duration of treatment	n of patients with complete or partial response to treatment	Potential biomarkers	Blood	Significant association between levels of biomarkers and response to treatment	Significant association in levels of biomarkers between CSU and controls		Significant association between levels of biomarkers before and after treatment	Country	Age, y	Design	Ref
											Before treatment	After treatment					
Romano et al.	2015	6	–	Omalizumab	150-300 mg	Every 2-4 weeks for 4-78 months	4	Tryptase	S	–	–	–	No	Italy	>16	R	[102]
Rasool et al.	2015	192	130	Subgroup a: VD, b: AH+CS, c: VD+AH+CS	VD: 60,000 IU per week (a, c)	4-6 weeks	–	Vitamin D	S	–	↓	–	Yes, ↓/↑ (a, c); No (b)	India	>16	P, CC	[103]
Oguz Topal et al.	2015	58	45	VD	300,000 IU/month	3 months	All	Vitamin D	S	–	↓	–	–	Turkey	<16>	P, CC	[104]
Asero	2015	29	–	Ciclosporin	3-5 mg/kg/d	3 months	25	D-dimer	P	Yes, ↓	–	–	–	Italy	<16>	P	[105]
Yalcin et al.	2014	8	–	Omalizumab	225-375 mg	12 months	–	D-dimer	P	–	–	–	Yes, ↑/↓	Taiwan	<16>	P	[106]
Altrichter et al.	2014	21	–	Omalizumab	75-375 mg	Every 2 or 4 weeks for 24 weeks	15	IL-31	S	Yes, ↓	–	–	Yes, ↑/↓	Germany	–	P	[63]
Rorie et al.	2014	42	–	VD in combination with nsAH	600- 4,000 IU/d	12 weeks	–	Vitamin D	S	–	–	–	Yes, ↓/↑	USA	>16	P	[107]
Basak et al.	2014	38	–	nsAH (loratadine or cetirizine)	10 mg/d	1 week	–	SCF, NPY, CGRP, NGF, VIP, SP	S	–	–	–	Yes, ↑/↓ NPY, VIP, SCF, NGF; ↓/↑CGRP; No (SP)	Turkey	>16	P	[61]
Boonpiyathad et al.	2014	50	40	VD in combination with nsAH	20,000 IU/day	6 weeks	–	Vitamin D	S	–	↓	↑	Yes, ↓/↑	Thailand	>16	P, CC	[62]
Puxeddu et al.	2013	4	61	nsAH	5-10 mg/d	6 weeks	–	sVCAM-1, sICAM-1	S	–	↑	–	No	Italy	<16>	P, CC	[108]
Asero	2013	91	–	nsAH	10-30 mg/d	1-2 weeks	83	D-dimer	P	Yes, ↓	–	–	–	Italy	<16>	P	[7]

Ohtsuka	2010	15	147	Ciclosporin	3 mg/kg/d	2-10 weeks	15	CRP	–	–	–	–	Yes, ↑/↓	Japan	>16	P	[109]
Inaloz et al.	2008	27	24	Ciclosporin	2.5 mg/kg/d	4 weeks	–	IL-2R, IL-5, TNF	S	–	↑IL-2R, TNF, IL-5	No (IL-2R, TNF)	Yes, ↑/↓IL-2R, TNF, IL-5	Turkey	>16	P, CC	[110]
Caproni et al.	2006	36	10	nsAH (levocetirizine)	5 mg/d	6 weeks	–	ELAM-1, ICAM-1, VCAM-1, P-selectin	S	–	↑ELAM-1, P-selectin	No (all)	Yes, ↑/↓ELAM-1, P-selectin; No (ICAM-1, VCAM-1)	Italy	>16	P, CC	[111]
Ojetti et al.	2001	33	25 ¹	HP eradication	Triple therapy	1 week ²	17	ECP	S	–	↑	–	No	Italy	>16	P, CC	[112]
Guida et al.	2000	10	6	Histamine-free diet	–	21 days	6	DAO, Histamine	P	–	↑Histamine	–	Yes, ↑/↓ histamine; No (DAO)	Italy	>16	P, CC	[113]

¹dyspeptic controls; ²ECP was evaluated at enrolment and 8 weeks after eradication; – not defined or no data; P – plasma; S – serum; VD – vitamin D; AH – sedative antihistamines; nsAH – non-sedating antihistamines; CS – corticosteroids; SCF – stem cell factor; NPY – neuropeptide Y; NGF – nerve growth factor; VIP – vasoactive intestinal peptide; CGRP – calcitonin gene-related peptide; SP – substance P; CRP – C-reactive protein; IL – interleukin; TNF – tumor necrosis factor alpha; DAO – diamine oxidase; ECP – eosinophil cationic protein; R – retrospective study; P – prospective study; CC – case-control study

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