

# Biomarkers In Chronic Spontaneous Urticaria: Current Targets And Clinical Implications

This article was published in the following Dove Press journal:  
*Journal of Asthma and Allergy*

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**Abstract:** Chronic urticaria (CU) is a mast cell-driven disease characterized by the development of wheals, angioedema, or both for more than 6 weeks. The two major sub-types are chronic spontaneous urticaria (CSU) and inducible urticaria. In the last decade different pathophysiological mechanisms, potentially responsible for the development of the disease, have been described. It is likely that the activation of mast cells and basophils in CSU can be the results of immune system dysregulation, activation of the inflammatory cascade, and of the extrinsic coagulation pathway. Some of the mediators involved in the pathophysiological mechanisms of CSU have recently been identified as potential biomarkers useful for the diagnosis, follow-up, and management of the disease, even if they are not yet available in clinical practice. Thus, in this review we discuss new insights in the mediators involved in the pathogenesis of CSU, highlighting their potential role as biomarkers in the activity and progression of the disease and response to therapies.

**Keywords:** chronic urticaria, inflammation, biomarkers, angiogenesis

## Introduction

Chronic urticaria (CU) is a common disease impacting negatively on multiple aspects of patients' lives and its management is often problematic for both the doctor and the patient. According to the recent guidelines, CU is defined as a disease characterized by the development of recurrent itchy wheals and/or angioedema occurring for more than 6 weeks, and it is divided into two major sub-types: chronic spontaneous urticaria (CSU) and inducible urticaria.<sup>1</sup>

In the last decade, the research has focused on defining the mechanisms underlying the development of CSU, identifying those mediators potentially useful as biomarkers for determining the severity and predicting the evolution of the disease and response to therapies. Skin mast cells are the main effector cells in the pathogenesis of CSU, but the underlying causes of their activation are largely unknown.<sup>2</sup> Autoimmunity seems to be involved in the pathogenesis of CSU, following the detection in a sizable subgroup of patients of circulating IgG autoantibodies directed to thyroid antigens and/or to IgE or to their high-affinity IgE receptor (FcεRI).<sup>3,4</sup> However, the observation that in a large part of CSU autoantibodies are not detectable suggests that other mechanisms are probably involved in the pathogenesis of the disease, thus opening the way to the identification of biomarkers beyond autoantibodies.<sup>5</sup>

## Immunological Biomarkers

It is well known that mast cells are the main effector cells in the pathogenesis of urticaria through the release of their pre-formed and newly synthesized mediators.<sup>2,6</sup>

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Although the triggers of mast cells activation in CSU are largely unknown and remain to be identified, several studies have allowed to identify in subgroups of CSU patients IgG autoantibodies directed to thyroid peroxidase (TPO) and/or to IgE or to their high-affinity IgE receptor (FcεRI).<sup>7,8</sup> Evidences of the role of autoimmunity in CSU stems from the observation that patients with CSU often have autoimmune disorders, including autoimmune thyroid disease.<sup>9</sup> Levels of IgG anti-TPO antibodies were detected in up to 33% of CSU patients and have also been proposed as promising biomarkers for the course of CSU, since their presence is associated to a longer duration of the disease.<sup>10</sup> Besides IgG anti-TPO, high levels of IgE anti-TPO antibodies have been recently detected in a subpopulation of CSU patients in which they might trigger mast cells and basophils degranulation binding to circulating antigens released after autoimmune thyroid damage.<sup>4,11,12</sup> However, the mechanisms whereby autoantibodies are involved in the development of CSU are still under investigation. So far, different mechanisms have been proposed.<sup>9,13</sup> A role of C5a in augmenting IgG-dependent histamine release from basophils and mast cells in CU has been demonstrated in in-vitro experiments,<sup>14,15</sup> suggesting a role of complement components in mast cells and basophils degranulation in CSU.

Using array analyses, more than 200 IgE autoantigens, either soluble or membrane-bound, were recently found in patients with CSU but not detected in control subjects. In particular, IgE anti-IL-24 were found in all patients with CSU, and showed an association with more severe disease, as proven by the urticaria activity score (UAS), and with a decrease in basophils count.<sup>16</sup> While there are several evidences of the role of IgE anti-TPO in CSU, the role of total IgE level is still not well defined, even if interesting data support their usefulness as biomarker of response to anti-IgE therapy with omalizumab.<sup>17</sup> In fact, a reduced efficacy of omalizumab has been reported in those patients with low level of total IgE.<sup>18,19</sup> Furthermore, basal levels of total IgE seem to correlate with time to relapse of CSU after discontinuation of omalizumab treatment. Interestingly, data from a small group of CSU patients suggest that the number of circulating basophils and their activity could be related to the clinical features of the disease. In fact, a negative linear correlation between basophil numbers and UAS has been observed in untreated CSU patients,<sup>20</sup> probably due to the recruitment from the circulation to the skin lesions.<sup>21,22</sup> Furthermore, analysis of basophil surface receptors demonstrated a link between the percentage of CD203c-expressing basophils and

severity of the disease.<sup>23,24</sup> Basophil CD203c expression turned out to be higher in severe than in non-severe CSU, suggesting that the quantification of basophil activation by measuring CD203c may be used as potential predictor of severe CSU.<sup>12</sup> Interestingly, the lack of basophil CD203c-upregulating activity in the serum of patients with CSU correlates with the clinical response to omalizumab therapy and modulation of the basophil FcεRI expression seems to contribute to the clinical improvement of CSU patients during omalizumab therapy.<sup>25,26</sup> Indeed, in CSU higher levels of basophil FcεRI expression have been observed in those patients with good response to omalizumab and their baseline levels seem to correlate with time of response to therapy,<sup>27</sup> suggesting its involvement as predictor of response to omalizumab.<sup>27,28</sup>

The involvement of the immune system in the pathogenesis and evolution of CSU depends not only on the production and activity of autoantibodies, but also on the activation of the complement system, since their components are able to induce mast cells activation.<sup>15,29</sup> In limited studies on the role of the complement system in CSU, it has been reported that C3 and C4 levels are significantly increased in CSU patients but not in healthy subjects and that their levels significantly correlate with C-reactive protein (CRP) in CU patients, but not in healthy subjects.<sup>29</sup> However, the increase of C3 and C4 levels has been observed only in a minority of severe disease (up to 5–10%), limiting their clinical usefulness as biomarkers of disease activity.<sup>29</sup> Importantly, elevated levels of C3, similarly to CRP, might strongly predict the cardiometabolic risk in patients with CU, especially in the severe and long-lasting disease.<sup>30</sup> We can also hypothesize that the increase of C3 and C4 levels is probably due to an augmented liver synthesis in response to pro-inflammatory cytokines, such as IL-1β, IL-6 or tumor necrosis factor (TNF).<sup>31</sup> Furthermore, the anaphylatoxin C3a, which is able to stimulate secretion of various pro-inflammatory cytokines and chemokines, is expressed on different cells associated with urticarial inflammation, including mast cells, basophils, eosinophils, neutrophils, monocytes, and is able to induce histamine release from skin mast cells with consequent vasodilatation and increased permeability of small blood vessels.<sup>32,33</sup> Parallel to C3a, it seems that C5a is required for activation of mast cells in CU by an IgG-dependent mechanism.<sup>15</sup> Taken together, these results support the role of the complement system in the pathogenesis of CSU directly, by inducing mast cell degranulation, or indirectly through amplification of the autoimmune

process. Even if the role of anaphylatoxins in the pathogenesis of CSU has been demonstrated, their usefulness as biomarkers in CSU still needs to be proven.

According to the recent guidelines of urticaria, the only generally available tests to screen for autoantibodies against either IgE for FcεRI are the autologous serum skin test (ASST) and basophil activation test (BAT).<sup>1</sup> In particular, the ASST is a non-specific test useful for evaluating the presence of any type of triggers of mast cells and basophils degranulation, including autoantibodies. In fact, a correlation between a positive ASST and the presence of IgG anti-FcεRI and anti-IgE antibodies was reported.<sup>34,35</sup> A positive ASST has been linked to an active disease and a reduced basophil count,<sup>36–39</sup> in addition, it seems that the positivity of ASST might be a good predictor of well-controlled CU during the 6-month step-wise treatment.<sup>40</sup> In parallel, positivity of ASST seems to predict a slow response to omalizumab.<sup>41</sup>

Taken together, all these results suggest that the ASST may be a useful tool for predicting response to treatment and monitoring therapeutic response in patients with CU,<sup>40,42–44</sup> but further studies are required.

## Inflammatory Biomarkers

Several independent studies performed in CSU patients demonstrated an important role of inflammation in the pathogenesis and clinical features of the disease. Recently, CRP has been proposed as a biomarker of CSU activity and/or response to treatment. Several clinical studies showed an increase of CRP in CSU patients, especially in patients with positive ASST with a strong correlation with other inflammatory markers (ie, erythrocyte sedimentation rate (ESR), blood leukocyte/neutrophil counts and IL-6 serum levels) and with disease activity.<sup>23,45,46</sup> Interestingly, in the complex scenario of the personalized medicine, CRP has also been proposed as a biomarker of response to therapy. According to recent studies, high levels of CRP may predict poor response to antihistamines and good response to oral CsA.<sup>45,47,48</sup> However, we have to take in account that the levels of CRP in CSU patients are quantitative lower than those detected in other inflammatory or autoimmune diseases where it is currently used as disease biomarker. Among the pro-inflammatory mediators, IL-6 seems to be a promising biomarker in CSU, due to its pivotal role in promoting inflammatory responses via activation of IL-6 receptor. Higher levels of IL-6 were detected in CSU patients in parallel to CRP, and its concentration seems

to discriminate between moderate/severe and mild disease, and between the active and the remission phase of urticaria.<sup>46,49</sup> Whether circulating IL-6 contributes to the pathogenesis of CSU or is merely a secondary consequence needs to be clarified. However, a potential use of this cytokine as a biomarker of disease activity can be hypothesized. Besides IL-6, other IL-1 family cytokines such as IL-18 have been proposed to play a role in the scenario of the pathogenesis of CSU. Most but not all the studies showed an increase of IL-18 in the circulation of CSU patients with limited information regarding its role in the disease activity.<sup>50–52</sup> In parallel to IL-1 family cytokines, a pathogenic role of the IL-23/IL-17 axis and TNF-α in CSU has also been hypothesized. IL-17A-producing CD4 T cells (Th17) are crucially involved in the pathogenesis of numerous autoimmune diseases. In particular, these cells have been identified on the basis of their ability to produce also IL-17F, IL-21, IL-22, IL-6 and TNF-α and are critically dependent on the pro-inflammatory cytokine IL-23 for their maintenance and survival. TNF-α is produced by skin mast cells and other inflammatory cells found at the site of urticarial lesions,<sup>53</sup> supporting its role in the pathogenesis of the disease. Since increased levels of IL-17, IL-23 and TNF-α were detected in CSU patients where they strongly correlate with the disease activity score, their potential role as biomarkers for disease activity has been also suggested.<sup>54</sup> IL-31, produced primarily by activated Th2 lymphocytes, skin-homing CD45RO CLA<sup>+</sup>T cells and mast cells, plays an important role in the induction of chronic skin inflammation. Higher levels of serum IL-31 were detected in CSU patients without any correlation with disease activity.<sup>55</sup> However, it has been observed that following treatment with omalizumab their levels significantly decreased, suggesting its potential role as a biomarker of response to omalizumab.<sup>56</sup> In the scenario of inflammatory mediators involved in the pathogenesis and clinical setting of CSU, an imbalance between pro- and anti-inflammatory adipokines has been proposed. Indeed, an increase in the levels of lipocalin-2 (LCN2), in parallel to a reduction of adiponectin, was reported in CU patients.<sup>57</sup> Interestingly, further studies demonstrated that the levels of LCN2 inversely correlated with the activity of the disease and patients with high LCN2 levels were those who responded to antihistamine therapy. Taken together, these results highlight the potential usefulness of the pro- and anti-inflammatory components of adipokines as biomarkers not only for disease activity, but also for the clinical response to specific therapies.

## Biomarkers Of Angiogenesis, Coagulation And Vascular Dysregulation

Angiogenesis is the growth of new blood vessels from preexisting ones. It is a multistep and highly orchestrated process involving vessel sprouting, endothelial cell migration, proliferation and tube formation.<sup>58</sup> Under physiologic conditions, angiogenesis depends on the balance of positive and negative angiogenic mediators within the vascular microenvironment and requires the functional activities of several mediators, including angiogenic factors, extracellular matrix (ECM) proteins, adhesion molecules, and proteolytic enzymes.<sup>58</sup> Angiogenesis is primarily involved in physiological processes, but it plays an active role in several pathological conditions, such as chronic inflammation, fibrosis, and tumor growth.<sup>59</sup> Several pro-angiogenic mediators have been identified. Among them, VEGF is the most potent pro-angiogenic mediator and its expression is often increased in chronic inflammatory diseases. Recently, the presence of new blood vessels in the skin of CSU patients has been reported.<sup>60</sup> The lesional skin contained significantly more CD31-positive endothelial cells compared to the normal skin and increased vascularity was also confirmed by confocal imaging, using the lectin *Ulex europaeus* agglutinin 1. The formation of new vessels as well as the increased number of eosinophils, neutrophils, basophils, and macrophages in the skin suggest a direct contribution of these inflammatory cells in the formation of blood vessels.<sup>61</sup> High levels of VEGF have been observed in the circulation and in the tissue of CSU patients,<sup>61</sup> suggesting its direct effect on vascular leakage as well as on new blood vessels formation. We can also hypothesize that mast cells and infiltrating eosinophils and basophils often present in skin lesions of CSU patients might contribute to the release of VEGF with consequent increase in vascular permeability. However, mast cells, eosinophils, and basophils are not only a source of VEGF, but they might be targets of this pro-angiogenic mediator, leading to perpetuation and amplification of inflammatory processes.<sup>62</sup> The functional activity of VEGF is tightly regulated by endogenous anti-angiogenic mediators mainly produced by the degradation of ECM components such as endostatin (ES) and thrombospondin-1 (TSP-1). We have recently reported increased levels of ES and TSP-1 in the sera of CSU patients, which do not correlate with the activity of the disease.<sup>63</sup> Thus, these anti-angiogenic mediators, able to exert multiple activities

during inflammation and angiogenesis, might be involved in the pathogenesis of CSU. We have to take into account that besides their anti-angiogenic activities, ES and TSP-1 also exert other important roles in the skin. For example, TSP-1 destabilizes a contact between endothelial cells due to its direct effect on the cells, contributing to skin vasodilation and consequent extravasation. ES, a proteolytic fragment of collagen type XVIII, acts as a vasoactive mediator due to its direct effect on endothelial cells via NO synthesis.<sup>64,65</sup> Therefore, both ES and TSP-1 might contribute to the vascular leakage in the skin of CSU patients, leading to the development of its clinical manifestations, such as wheals and flare formation. Since these mediators, although increased in the serum of CSU patients, do not correlate with disease activity or other clinical parameters, they cannot be considered biomarkers of the disease. Parallel to ECM components, some members of MMPs were observed to be increased in the circulation of CSU patients.<sup>23</sup> Among them, MMP-9 is increased in the peripheral blood of CSU patients, both adults and children, and it is suggested that its contribution to CSU pathogenesis is probably due to its direct effect on tissue remodeling.<sup>23,66,67</sup> Furthermore, MMP-9, produced by several inflammatory cells, including macrophages, neutrophils, T cells, and mast cells, promotes migration and activation of immune cells by cleaving pro-inflammatory chemokines and cytokines and therefore contributing to the inflammatory processes.<sup>68,69</sup> However, some but not all studies showed an association between plasma levels of MMP-9 and disease activity.<sup>23,67,70</sup> Therefore, further studies are required before considering MMP-9 as biomarker in the activity and progression of CSU.<sup>26</sup> During angiogenesis, VEGF also promotes an increased expression of leukocyte adhesion molecules, such as ICAM-1, VCAM-1, and E-selectin.<sup>71</sup> The soluble forms of ICAM-1 and VCAM-1 are widely used as biomarkers of endothelial dysfunction and their increase in the circulation and skin biopsies of patients with CSU seems to reflect a pro-inflammatory endothelium phenotype.<sup>22,72,73</sup> The enhanced expression of VCAM-1 and ICAM-1 is involved in pathogenesis of wheal, since they induce augmented permeability of vessels. Furthermore, an increase of the soluble form of endothelial (VE)-cadherin (sVE-cadherin), an endothelial cell-specific adhesion molecule located at the junction between endothelial cells, has been reported in patients with CSU.<sup>74</sup> Moreover, serum levels of sVE-cadherin were closely associated with the severity of the disease, suggesting that this soluble adhesion



molecule might be useful for evaluating the severity of CSU.<sup>74</sup> Since histamine can induce VE-cadherin phosphorylation, increased sVE-cadherin levels might, at least in part, be a consequence of histamine release and antihistamine therapy might attenuate histamine-induced VE-cadherin damage.<sup>74</sup>

Recently, the detection of increased levels of factor VIIa, prothrombin fragment 1+2, and D-dimer in CSU patients suggests that, following endothelial cell activation, tissue factors are released with consequent activation of the extrinsic coagulation cascade and secondary fibrinolysis.<sup>37,75,76</sup> These results are of particular interest considering that thrombin can increase vascular permeability and is a potent inducer of mast cell degranulation, at least in experimental models.<sup>77</sup> Furthermore, the levels of prothrombin fragment 1+2 and D-dimer seem to correlate with the activity of the disease. In particular, the level of D-dimer significantly correlates with UAS in CSU as well as in acute urticaria, suggesting its role as a marker of disease severity in both forms of urticaria.<sup>78–81</sup> Evidences for a possible association between the levels of D-dimer and CRP and between these mediators and disease activity are still limited. Thus, it is possible to hypothesize a role of activation of the coagulation system and inflammation in CSU pathogenesis,<sup>82</sup> but it has not yet been demonstrated that the increase in the levels of D-dimer is restricted to the CSU rather than due to comorbidities common in patients with CSU, such as chronic infections and autoimmune disorders.<sup>66</sup> Recently, D-dimer has been proposed as a marker of resistance to antihistamine therapy, response to omalizumab treatment and a useful tool to monitor clinical response to CsA.<sup>37,48,83,84</sup> Regarding the usefulness of the D-dimer detection during omalizumab therapy, conflicting results have been reported by Marzano et al who have not confirmed the link between D-dimer and response to omalizumab treatment in CSU.<sup>85</sup>

Since higher mean platelet volume (MPV) is associated with inflammatory conditions, MPV has been explored as biomarker of disease activity in CSU.<sup>86,87</sup> In particular, the severity score of CU was significantly correlated to the MPV in patients with positive ASST, but not in those with negative ASST.<sup>39</sup> It has been hypothesized that MPV plays a role in the inflammatory processes underlying CSU and it might be a marker of disease activity.<sup>37</sup> However, these promising results were not confirmed in other studies.<sup>88</sup> A higher platelet count was instead found in CSU patients with more severe disease activity, positively related with serum CRP concentration, suggesting that acute phase response in CSU is

associated with the increased number of circulating platelets in patients with more severe symptoms.<sup>88</sup>

## Clinical Biomarkers

Several studies have demonstrated the usefulness of different clinical markers of disease severity, response to therapies and remission of the disease in CSU.<sup>89</sup> Some data showed how age could be inversely associated with disease severity and improvement rates are significantly higher in patients <19 years old as compared to adults.<sup>89–91</sup> Furthermore, it seems that the age of CSU patients significantly predicts the quality of life impairment assessed with the disease-specific quality of life instrument CU-Q2oL. In particular, it has been observed that younger patients were more affected on functioning and itching/embarrassment, whereas older patients were more severely affected on sleep and swelling/eating.<sup>89</sup> Epidemiological studies showed that most CSU patients are females between 20 and 59 years old and being female seems to be a predictor of longer time of remission and quality of life impairment.<sup>89,92,93</sup> Although studies are limited, it appears that sex hormones could play a role as triggers of the disease in a small subset of patients.<sup>94</sup> Most of the women studied did not experience CU before puberty; worsening symptoms was observed in a small group of pregnant women during urticaria, and hormonal contraception was associated with aggravation of symptoms in a minority of women.<sup>94</sup> Furthermore, a decreased serum concentration of dehydroepiandrosterone sulphate (DHEA-S) was observed in CSU patients, with levels significantly lower in symptomatic patients than in disease remission.<sup>95,96</sup> Currently, it is not clear if a neuroendocrine-immune dysfunction, due to psychological stress, contributes directly to CSU pathogenesis or it is indeed an epiphenomenon: thus, DHEA supplementation as adjuvant therapy for the disease cannot be recommended. The duration of the disease has been also proposed as marker of severity.<sup>10,92</sup> In particular, the improvement of urticaria during treatment has been reported in patients with duration <1 year compared to a longer period.<sup>89</sup> The duration of the disease was also correlated with the presence of angioedema and the positivity of anti-thyroid antibodies.<sup>10</sup> In particular, the presence of angioedema is identified as a factor strictly related to a less favorable prognosis since CSU patients who show concomitant angioedema have longer disease duration and time to remission.<sup>10,89</sup> In around 10–50% of CSU patients, the association with inducible urticaria leads to a longer disease duration with remission rates observed only in 21% after 1 year of the disease compared to 47% in patients without angioedema.<sup>89</sup>

Potential markers of CSU have also been investigated in patients with co-morbidities. Among them, arterial hypertension has been associated with long duration of urticaria and patients with CSU are characterized by an increased risk of development of hypertension.<sup>89</sup> Recently, in Korean patients with CU and concomitant metabolic syndrome (MS) the levels of eosinophil cationic protein (ECP), TNF $\alpha$ , and the complement components C3 and C4 were significantly higher than those in CU without MS.<sup>97</sup> Prevalence of MS is significantly greater in patients with CSU than in healthy controls and it seems to be an independent predictor of uncontrolled CSU.<sup>89</sup> Therefore, patients with severe and uncontrolled CSU should be evaluated for a concomitant MS in order to reduce their cardiovascular risk and to improve the urticaria outcomes. It is still unclear whether the increase of systemic inflammation is just an epiphenomenon or it might play a role in the pathogenesis of CU.<sup>97</sup> Furthermore, exacerbations of CU following aspirin or NSAIDs intake have been associated with more severe CU and its duration.<sup>89,98,99</sup> As observed in other inflammatory and autoimmune diseases, the lack of response to treatment in CSU is a relevant marker of severity of the disease. Increasing evidences demonstrate how the failure to respond to high-dose anti-histamines, up to four times the usual dose as indicated by recent guidelines,<sup>1</sup> is related to more severe disease.<sup>99</sup> Those patients not responding to anti-histamine therapy usually require omalizumab or other immunosuppressive agents as a second-line treatment. Actually, specific markers that may help to predict a poor response to first-line therapy have not been identified. Some data suggest that increased complement C5a fraction, elevated D-dimer plasma levels, positivity of the ASST or BAT, as well as other clinical indicators of severity, may be potential markers of response to first-line therapy.<sup>44,99,100</sup> In patients under omalizumab treatment, a significant correlation between response to therapy a positive Basophil Histamine Release Assay (BHRA) has been observed.<sup>101</sup> In those patients under CsA therapy, it has been demonstrated a positive correlation between response to therapy and BAT, BHRA and ASST but a negative correlation with high levels of D-dimer.<sup>84,102–104</sup> In particular, the basal D-dimer levels have been proposed as useful marker for monitoring the clinical response to CsA treatment.<sup>78</sup>

## Novel Biomarkers

Growing interest has recently been given to the possibility to identify new biomarkers useful to predict the future

evolution and response to treatments or to monitor the activity of CSU and the efficacy of treatment.

Recently, the complex role of heat shock proteins (Hsp) in inflammation and immunity has been objective of attention, due to their ability to promote cytokines production and adhesion molecules expression and to play modulatory roles in both humoral and cell-mediated immune responses, as well as in the complement activation. Among Hsp, increased circulating levels of Hsp70 and anti-Hsp70 antibodies were detected in CSU patients,<sup>105</sup> however the mechanism and clinical significance of this observation in CSU remain to be clarified. Hsp70 seems to exert a protecting role, by promoting anti-inflammatory immunoregulatory T-cell responses, and in parallel to exert a pro-inflammatory effect, by activating NF- $\kappa$ B pathway and IL-6 signaling system. Since Hsp may act as an autoantigen, it has been hypothesized that Hsp70 triggers both cell-mediated and humoral immune responses in CSU and anti-Hsp70 antibodies may activate pro-inflammatory processes. In addition, patients with moderate-severe CSU show higher plasma Hsp70 concentration and higher anti-Hsp70 antibodies levels, although no significant difference was identified between moderate-severe and mild disease activity.<sup>105</sup> Thus, the potential pathogenic role of Hsp70 and anti-Hsp70 antibodies in CSU and their usefulness as biomarkers remain to be elucidated by further investigations.

It has also been proposed that oxidative stress contributes to CSU pathogenesis by modulating enzymatic function and by inducing pro-inflammatory cytokines release. Their effects seem to be the results of an imbalance between ROS production and the effectiveness of antioxidant defense mechanisms. Nettis et al have recently investigated the role of advanced glycation end products (AGEs) and advanced oxidation protein products (AOPPs) as new potential oxidative stress biomarkers in CSU, reporting levels of AOPPs, but not that of AGEs, significantly higher in CSU patients.<sup>106</sup>

A fundamental contribution in understanding the mechanisms of gene modulation comes from epigenetic studies, conducted recently also in skin diseases, including urticaria. This field deals in particular with inheritable and potentially reversible modifications of DNA which can modulate gene expression without altering DNA sequence and which may be influenced by environmental factors. In this context, microRNAs (miRNA) represent an important class of small non-coding RNAs, that induce gene silencing by binding to target sites of the targeted messenger RNA. Growing interest has recently been given to the

involvement of miRNA in skin disorders because of their potential ability to influence regulatory mechanisms of inflammation. Five miRNAs (2355–3p, 4264, 2355–5p, 29c–5p, and 361–3p) were found to be significantly increased in CSU. Thus, these five miRNAs, which target genes associated with several cellular functions such as cellular motility, regulation of leukocyte migration, and inflammatory response, have been suggested as biomarkers for chronic autoimmune urticaria.<sup>107</sup>

Furthermore, the expression of upregulated or down-regulated miRNA was recently investigated in CSU patients and the data were then validated by reverse transcription qPCR. The expression of miR-125a-5p and CCL17 levels were found to be significantly increased in the sera of these patients and significantly decreased in the remission phase of the disease, suggesting a potential use of miR-125a-5p and CCL17 as biomarkers of activity of CSU.<sup>108</sup>

In the last few years, great attention has been given to the immunomodulatory activity of vitamin D, which have potential clinical implications in the susceptibility to autoimmune and allergic diseases.<sup>109,110</sup> Furthermore, vitamin D was also proposed as a biomarker of CSU based on the detection of low levels of vitamin D in patients with CSU compared to those with acute urticaria and atopic dermatitis.<sup>111</sup> On the basis of these observations, the beneficial effect of vitamin D supplementation in the course of CSU has been proposed, but its usefulness as a biomarker of the disease has yet to be demonstrated.

## Conclusion

In the last year, several studies have been published in order to better understand the pathogenic mechanisms underlying CSU and to identify potential biomarkers of both disease activity and response to therapies (Table 1). Several cells of the immune system, soluble mediators, adhesion molecules, autoantibodies, complement and coagulation system components contribute to mast cells and basophil activation and degranulation, leading to the development of CSU. Many of the above-mentioned cells and mediators seem to be involved in the pathogenesis of the disease, but none of these seems to be really specific to CSU. Several of these mediators correlate with the UAS, suggesting their potential use as biomarkers of disease activity. Furthermore, some clinical aspects of CSU and co-morbidities have been proposed as potential biomarkers of duration of the disease

**Table 1** Clinical And Molecular Biomarkers In CSU

Type of Markers	Markers	References
Disease activity	Basophil count	20
	CD203-c basophils	24
	CPR	23,45,46
	IL-6	46,49
	IL-17, IL-23, TNF-α	54
	LCN2	57
	Prothrombin fragment 1+2	75,81
	D-dimer	75,78,80
	NSAIDs exacerbation	89,98,99
Disease course	ASST positivity	36,37
	IL-6	46,49
	IgG anti-TPO	10
	Age	89,90
	Gender	89,92,93
	Angioedema	10,89
	Association with inducible urticaria	89
	NSAIDs exacerbation	89,98,99
	Metabolic syndrome	89
	Basophil FcεRI expression	27,28
Response to therapy	CPR	45,47,48
	IL-31	56
	LCN2	57
	D-dimer	13,37,83,84,100
	C5a	43
	ASST positivity	37,38,40,44,102,103
	BHRA positivity	101,102,104

and response to therapies. However, these recent data, even if promising, need future validation before being used in clinical practice.

## Disclosure

The authors declare no conflicts of interest in this work.

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