

Biomarkers to Diagnose, Assess and Treat Chronic Spontaneous Urticaria: Not There Yet

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Opinion statement

Purpose of Review Biomarkers to identify patients with chronic spontaneous urticaria (CSU), to grade clinical severity of CSU, and to guide therapy selection in patients with CSU have not yet been validated in any large CSU patient population. As such, there is insufficient evidence to recommend routine use of any particular biomarker. This review will summarize ongoing research in the field of biomarker development and how it could 1 day benefit diagnosis, severity grading, and treatment decisions in CSU.

Recent Findings Second-generation antihistamines are the accepted first-line therapy in the treatment of CSU. The United States Food and Drug Administration (FDA) has approved omalizumab for use in CSU that is refractory to antihistamines. Multiple alternative pharmacologic therapies have been utilized with varying levels of success, although no others are FDA approved. Of the alternative therapies, cyclosporine is the best-studied. Hydroxychloroquine, sulfasalazine, dapson, and colchicine are others that are frequently prescribed. While oral corticosteroids can provide symptomatic relief for most patients, using corticosteroids for more than a couple of weeks is not advised due to significant side effects. Regardless of which therapy is chosen, a significant fraction of patients do not respond. With the emerging market of biologic products targeting specific immunologic molecules, additional therapies for CSU are likely to become available in the next 10 years. Identifying biomarkers

to assess disease response to therapy and to determine which patients are most likely to benefit from which therapy would greatly benefit the field.

Summary The ideal tissue source for biomarker identification in CSU has not been determined. Basophil trafficking has been demonstrated to be abnormal in CSU and would benefit from further exploration. Autoreactive T cells have been recently identified in some CSU patients and combined with autologous serum skin test may be useful as a diagnostic tool. Finally, relative to the search for blood biomarkers, the skin has been a poorly charted territory as a possible source for biomarkers and would likely benefit from additional investigation.

Introduction

Chronic spontaneous urticaria is clinically defined as 6 weeks or more of spontaneously occurring urticaria with or without angioedema. In 1865, Balmanno Squire, MB described:

"No disease is looked on as more hopeless than chronic urticaria . . . certainly common enough to render it most desirable that [its] origin and nature should be generally understood; and [it is] severe enough, both as regards the degree of distress [it] occasion[s] and the length of time over which [its] course usually extends, to make it advisable that some more efficient means should be employed for [its] relief than what [is] generally thought to be the appropriate remedies for [it]." [1]

Since his writing, "more efficient means" of treating chronic spontaneous urticaria (CSU) have arrived including non-sedating antihistamines and omalizumab as have many immune-modulating medications that are used off label [2••, 3••, 4, 5•]. While these newer medications are more targeted than therapies in the 1800s, none of them universally treat or eliminate disease. A major limitation to current research is the lack of an animal model for CSU. The history of key immunologic findings in CSU has recently been summarized [6]. Until the "origin and nature" of CSU has been pinpointed, treatment and cure will remain elusive.

Of the approximate 0.1–1% of the population thought to be affected by CSU at any given time [7], the majority, estimated to as high as 75%, will respond to treatment with non-sedating antihistamines at up to four times the conventional dosing [8–10]. Of patients refractory to this first-line therapy, 65–83% respond to omalizumab [11–13]. There are no FDA-approved alternatives for the remaining patients, although various anti-inflammatories and immunosuppressants have been used with varying levels of success [2••, 3••, 11, 14] and novel biologics are also being assessed for efficacy [15, 16].

At the core of identifying biomarkers that are useful to diagnose CSU, score severity and treat CSU are the questions: What mechanisms cause human cells to be disturbed enough to generate pruritus and hives? And why are some skin areas affected more than others? Various cell types have been implicated in the pathogenesis of CSU including basophils [17], mast cells [18], eosinophils [19•], platelets [20], and autoreactive T cells [21••], although to date no unifying mechanism has been described. The quest for biomarkers is therefore a quest also to better understand mechanism of disease. In this quest, peripheral blood specimens, skin sampling, and genetic analyses have been the three areas where research has been focused and how this review is also organized.

Potential Biomarkers in Blood

Blood biomarkers are highly sought because of the relative ease in obtaining serial measurements, minimal invasiveness, and the present standardization of a multitude of assays. In a recent systematic review by Kolkhir and colleagues, nine potential blood biomarkers were identified as having strong evidence to

distinguish CSU patients from healthy controls: tumor necrosis factor, D-dimer, C-reactive protein, factor VIIa, prothrombin fragment 1 + 2, dehydroepiandrosterone sulfate, vitamin D, mean platelet volume, and matrix metalloproteinase-9 [22••]. In the same review, IL-6, mean platelet volume, D-dimer, prothrombin fragment 1 + 2, and C-reactive protein were found to have strong evidence supporting their correlation with disease activity. (Prothrombin fragment 1 + 2, D-dimer, and C-reactive protein had also been found to be associated with disease severity in a previous review [23]). Each of these markers are unfortunately elevated in a variety of disorders, many of which can co-exist with CSU and therefore offer poor specificity in the diagnosis of CSU. Authors went on to demonstrate that available evidence is weak or non-existent for any biomarker to differentiate CSU from other disease entities and to prognosticate CSU disease outcomes or response to therapy.

Where might one begin looking for blood biomarkers to specifically diagnose CSU or to prognosticate outcomes or response to therapy? Basophil trafficking is a key area of biomarker research [24•]. Relative basopenia has been recognized as a common feature in CSU for decades; skin biopsy specimens from CSU patients routinely show basophils where they are not typically found in healthy controls, suggesting that basopenia occurs due to migration of basophils from blood to skin. When 114 patients presenting with acute spontaneous urticaria of unknown cause were prospectively followed, patients with the most profound basopenia were more likely to progress to CSU [25]. Basopenia improves with treatment of CSU and in natural remission [26, 27]. Post hoc analysis of pooled data from 3 large double-blinded randomized placebo-controlled clinical trials of omalizumab in CSU including 469 patients on omalizumab and 139 patients on placebo demonstrated that as patients' symptom scores improved so did their circulating basophil counts [28•]. What causes basophils in CSU to be deposited into the skin is still unknown. Prostaglandin D2 promotes chemotaxis of basophils via its receptor chemoattractant receptor-homologous molecule expressed on TH2 cells (CRTH2) and when this pathway is activated, the CRTH2 receptor is internalized [29•]. Patients with CSU had decreased CRTH2 surface receptor expression on their basophils as compared to healthy controls suggesting *in vivo* prostaglandin D2 exposure [29•] as a possible pathway leading to basophil recruitment.

Basophil activation and stimulation has also been explored in CSU [17]. Increased CD203c expression on basophils has been associated with CSU and recently higher CD203c expression has also been associated with increased CSU severity [30]. Serum from CSU patients often stimulates donor basophils to release histamine as evidenced by the presence of CD63 expression on basophil cell surfaces in indirect basophil activation testing (BAT), a test that has been proposed by many to be useful in distinguishing a subset of patients with autoantibodies to basophil surface IgE and/or FcεRI. Recently, the indirect BAT was further optimized for this purpose [31] and pediatric reference ranges were described for the BAT and CD63 expression above 3.4% was more than 80% specific in predicting which patients had moderate to high disease severity [32].

When looking directly at basophils from CSU patients (and not basophils from healthy controls stimulated with CSU serum), IgE-mediated histamine release is often decreased and this has recently been correlated with disease

activity [27]. What causes suppression of the IgE-mediated histamine release pathway in basophils in many CSU patients is unknown. The unidentified suppressive factor has been detected in serum of CSU patients, is present during disease, reduced during remission, and is not dependent on IgE and IgG [33]. Identification of this factor may lead to a promising biomarker.

The relatively quick remission of CSU, averaging a few days, in a proportion of patients treated with omalizumab initially baffled many because the mechanism of action in allergic disorders such as asthma often requires weeks for noticeable clinical improvement [34]. Recently, Gericke and colleagues demonstrated that patients with a positive basophil histamine release assay (BHRA), an assay commonly used for the detection of serum autoantibodies against the unoccupied Fc ϵ RI receptor or cell-bound IgE, were more likely to be slow responders, averaging weeks until remission, than patients without evidence of these autoantibodies [35•]. Those with a negative BHRA were more likely to respond in an average of a few days, suggesting that omalizumab is working via two separate and important mechanisms. However, there was some overlap in the response of these BHRA subsets.

Consistent with the BHRA testing, differences in serum testing in a different basophil assay have been noted between patients who do and do not respond to omalizumab. CSU patients whose sera failed to upregulate CD203c in basophils obtained from healthy controls were more likely to respond to omalizumab than their counterparts whose sera did upregulate CD203c [36]. MacGlashan and Saini recently identified increased intracellular syk in peripheral blood basophils as a potential biomarker to predict which patients with severe asthma would respond to omalizumab [37•]. A similar study of intracellular syk levels in CSU may be useful in identifying optimal candidates for omalizumab therapy, but at baseline, basophil IgE function is altered in CSU [17].

An inflammatory process that is ongoing and separate from histamine release has been postulated [20] and the extrinsic coagulation pathway is increasingly recognized as a component of CSU pathogenesis [38, 39]. Although platelets can be activated by some factors including platelet activating factor that can also activate basophils and mast cells, platelet function in CSU has been assessed without a clear emerging pattern between CSU patients and controls [20, 40–42]. In 29 patients whose CSU was refractory to multiple daily doses of antihistamines, the majority demonstrated elevated D-dimer levels and these levels improved when clinical course improved with cyclosporine treatment [5•]. Similarly, in a study of 32 patients with CSU refractory to multiple daily doses of antihistamines, D-dimer levels improved with clinical response to omalizumab [43]. Elevated D-dimer has also been associated with increased CSU severity measured by the urticaria activity score (UAS) [44]. CRP has been noted to be elevated in CSU patients vs. controls [22••, 45] and to be correlated with severity of CSU [22••, 46, 47]. IL-6, an interleukin associated with the synthesis of CRP, is also elevated in CSU patients [48–50] and levels are further elevated with increased severity [49]. Pentraxin 3, locally produced at inflammation sites, is similarly elevated with severity in CSU as CRP [47]. Median levels of serum clusterin, a modulator of the complement system, were increased in a group of CSU patients vs. controls [51]. While measures of inflammation may one day be useful in monitoring disease severity in CSU, it is unlikely that these non-specific markers will be useful in aiding diagnosis.

What may be a promising biomarker for CSU diagnosis? Auyeung et al. discovered antigen-specific T cells in CSU for the first time [21••]. Interferon-gamma release resulting from T cell stimulation preceded autoantibody detection during natural history of CSU in observed patients. Looking in combination for the presence of either antigen-specific T cells or autoantibodies to FcεR1α demonstrated high sensitivity and specificity for diagnosis of CSU in this small patient cohort. Such a tool if validated in a larger population may prove useful in the diagnosis of CSU.

Additional studies have recently been performed on various markers that have been assessed in the past. Vitamin D has been implicated in CSU pathophysiology in most [52, 53] but not all studies [22••, 54, 55•]. Total IgE levels were elevated in 63% of patients with CSU in a study including 93 patients in a Southeast Asian skin allergy clinic [56]. Exploratory studies to assess for biomarkers have been performed on a variety of other substances measurable from peripherally collected blood specimens in CSU patients and additional follow-up may benefit the field. For example, in a couple of pilot studies, substance P was noted to be elevated in CSU serum [57, 58]. Plasma oxidative stress was notably elevated in children with CSU and also was more increased with increased disease activity [59]. Iron and B12 levels were marginally different between CSU patient and control populations CSU [54]. Histamine-releasing factor and histamine-releasing factor-reactive IgE levels were greater in patients with CSU than controls [60]. Elevated interferon lambda 1 levels were noted in the plasma of CSU patients but not healthy controls or patients with atopic dermatitis [61]. Total serum tryptase is elevated in some patients diagnosed with CSU [62], and recently baseline serum tryptase levels were mildly elevated in patients with CSU who described systemic complaints beyond urticaria and angioedema in comparison to their counterparts without these complaints [63]. Elevated tryptase level was also associated with increased urticaria symptoms at time of blood draw [63].

In summary, a variety of assays on peripheral blood specimens from patients with CSU have been performed to ascertain whether those markers may be useful in treatment or management decisions in CSU. To date, several biomarkers have shown promise but no biomarker has been identified to be clinically useful in a large, diverse CSU population.

Potential Biomarkers in Skin

The skin has been much less explored than blood as a source for possible biomarkers in CSU. Of the research on skin parameters in CSU, the autologous serum skin test (ASST) has been the most reported. In a structured review of multiple studies, ASST positivity was associated with increased likelihood to have severe and/or prolonged disease [55•]. The meaning of a positive ASST test is unknown. ASST test results are not constant over time in patients with active disease and some convert from positive to negative or from negative to positive [25]. While some advocate for the splitting of patients into a category of autoimmune spontaneous urticaria [64], it is unclear how to segregate this patient population and what the clinical ramifications are for this diagnosed subset. In a prospective study of 92 children with CSU, remission rates did not

differ significantly between patients with positive ASST ($n = 37$) and those without [65]. In a retrospective study of biopsies from 37 patients with CSU including 18 who had detectable anti-IgE receptor antibody and 19 who had no detectable antibody, no significant microscopic difference was seen between groups [66], similar to findings from past work [67].

When skin is biopsied, elevated eosinophil and neutrophil counts are often seen in CSU wheals. Whether mast cell numbers are also increased in wheals of CSU depends on the reported study. Unfortunately, of the few CSU studies that include examination of patient biopsies, few are prospective in nature where the timing of the biopsy is specified in relation to wheal appearance, where treatment regimens are known and described, and where disease severity is delineated. Three such studies, although small, have recently been reported. In a biopsy study of eight patients with CSU and nine healthy controls, increased numbers of CD31+ endothelial cells and CD31+ blood vessels, two measures of vascularity, as well as eosinophils were seen in both involved and uninvolved skin of patients with CSU vs. controls [19•]. These markers were present at higher levels in wheals than in non-lesional skin. Mast cells were present at higher levels in patient vs. control biopsies, although these were not present at higher levels in wheals than in non-lesional skin. These same patients were used in another CSU biopsy study that revealed increased expression of calcitonin gene-related peptide, a potent vasodilator, and vascular endothelial growth factor expression in lesional skin of patients with CSU vs. non-lesional skin and healthy controls [68]. Put together, these studies suggest that increased vascularization is an integral part of CSU pathology and may be useful as a marker of disease activity. Finally, in a third study with the same patients and controls, CSU lesions were noted to contain more IL-33+, IL-25+, and TSLP+ cells than patients' non-lesional skin and healthy controls' skin [69]. As TH2-initiating cytokines, IL-33, IL-25, and TSLP's presence implicate TH2 skewing in the pathophysiology of CSU. Unique to each of these three prospective studies are that biopsies were timed to occur 4–8 h after appearance of a new wheal to approximate the timing when late-phase allergic skin reaction is present and histamine reaction has resolved.

Retrospective archived specimen studies from previously obtained biopsies have been reported more commonly. For example, biopsy specimens from 36 patients with CSU were analyzed in parallel with a retrospective chart review of these patients' clinical courses [70•]. The histopathologic variable that best predicted treatment refractory disease course was subcutaneous cellular infiltration. Features that were not significantly different between treatment responders and non-responders included edema, type of dermal infiltration, and number of infiltrated neutrophils or eosinophils. In another cross-sectional study of 41 patients with CSU who had biopsies at various time points and on various treatment regimens, 19 (46%) had a predominantly eosinophilic infiltrate and this subset of patients was more likely to have increased CSU severity using the UAS [71]. Finally, in a third recent biopsy study from retrospectively reviewed pathology specimens, 11 skin biopsies from 9 patients were examined and compared to uninflamed skin from patients having biopsies for other reasons [72]. Immunohistochemical staining was used to demonstrate that TH2 and TH17 cells were more common in skin from CSU patients than in controls and that TH1 and TH22 cell numbers were not significantly different between these two groups.

As evidenced by small patient numbers included in recent CSU biopsy publications, the labor involved in searching for biomarkers in the skin is great. Performing skin biopsies on large numbers of patients to validate clinicopathologic findings in CSU in pursuit of biomarker identification is difficult for multiple reasons including (1) skin biopsies are scarring, (2) a biopsy site can only be used once, representing only one point in time in a disease process where most lesions come and go over periods of 24 h with different phases; (3) biopsies must be obtained by adequately trained medical professionals, (4) biopsy results are not automated and require trained medical professionals for interpretation, and (5) using one biopsy from one location as representative of one patient's disease process relies on the assumption that biopsies performed on other lesions in other locations in other phases of development on a particular patient would look the same microscopically. Why then should the skin be assessed at all? And if it is assessed, are there simpler means to look for biomarkers from it?

To answer the first question, increasing evidence demonstrates unique immunologic cell populations including resident memory T cells that are present in the skin and do not circulate [73••]. Relying solely on peripheral blood collection and analysis removes the possibility of detecting these cell types that may be integral in generating or promoting inflammation seen in CSU. To answer the second question regarding whether simpler means of analyzing skin exist, a rapidly developing technology being explored as a diagnostic tool in other fields of medicine is hyperspectral imaging. In brief, hyperspectral imaging is a non-invasive and radiation-free technology that captures the visual spectrum with vaster depth than the human eye and allows for the separation of objects or object parts by physical differences that reflect an object's material make-up [74]. The idea that hyperspectral imaging may be useful in specifying endotypes of atopic dermatitis was recently described by Happel [75•]. Similarly, the possibility that hyperspectral imaging may be used as a biomarker to stratify patients with CSU into categories of who will benefit from which therapy should be explored. The benefits of any identified hyperspectral signal that could be used as a biomarker are multiple, including (1) being noninvasive it leaves no scar, (2) large area sampling is possible; unlike biopsy, sampling is not limited to a few specimens, (3) because no skin is removed, the same rash can be imaged over time.

In summary, several small studies involving skin biopsies have demonstrated differences between CSU patients and controls. Increased vascularity, increased inflammatory infiltration into subcutaneous tissue, and increased TH2 skewing have been demonstrated in some CSU patients vs. controls. While using serial skin biopsies as future biomarkers would be difficult, it is possible that rapidly emerging imaging technologies, particularly hyperspectral imaging, may be able to capture signals generated from these differences seen between CSU patients and controls that could lead to biomarkers for diagnosis, treatment, and/or severity grading.

Genetic Predictors

Current evidence does not point toward a common genetic mutation that could diagnose CSU or predict outcomes in the majority of patients. Several genetic

polymorphisms have been found in small patient subsets. Polymorphisms of *ORAI1*, a gene that encodes a calcium channel essential in mast cell degranulation, were discovered in the Han population of South China to be associated with CSU and with desloratadine efficacy [76]. Also in the Han population of South China, *FCER1A* genetic polymorphisms were associated with risk for CSU and decreased efficacy of H1 antihistamines [77] and a component 5a receptor 1 polymorphism was associated with increased risk for CSU and decreased response to desloratadine [78]. Genetic polymorphisms in interferon-gamma and IL-6 were associated with CSU in an Indian cohort [50]. A missense variant of *ADGRE2*, a gene encoding a subclass of G-protein coupled receptors, was found in three Lebanese families with an autosomal dominant inheritance pattern of vibratory urticaria [79]. MicroRNAs have also been explored for possible expression differences between CSU and control patients [80], although in order to be considered for future use as a biomarker, microRNA data would need better normalization to define normal populations.

Conclusions

Biomarkers to specifically diagnose CSU, to monitor disease severity, and to predict response to therapy would be highly useful for patient care. Many large and small exploratory studies looking for blood biomarkers have been performed with limited success to date. Possible blood biomarkers that may benefit from further exploration include the use of basophil intracellular syk for determining which patients will respond to omalizumab and the use of autoreactive T cells in combination with the ASST in diagnosing CSU. Exploration for possible biomarkers in the skin has been much more limited than exploration for possible biomarkers in the blood largely due to the limiting and scarring nature of serial human skin biopsies. Hyperspectral imaging is a rapidly evolving radiation-free, non-invasive technology that is being used in research studies to identify defining physical properties of skin lesions in other fields of medicine and the field of CSU may similarly benefit from research exploring its possible uses for biomarker identification.

Compliance with Ethical Standards

Conflict of Interest

Corinne Happel declares that she has no conflict of interest. Sarbjit Saini reports grants from Astra Zenca, grants and non-financial support from Novartis, other from Genentech, outside the submitted work.

Human and Animal Rights and Informed Consent

With regard to the senior author's research cited in this paper, all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. In addition, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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