The Role of Platelets in Chronic Urticaria

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Key Words

Chronic urticaria · Pathogenesis · Platelets

Abstract

Background: Platelets are implicated in many pathophysiological processes, including inflammation and immunity. Ever-growing evidence suggests the active involvement of platelets in the pathogenesis of various inflammatory disorders, including cutaneous inflammatory diseases. A limited number of studies have investigated the role of platelets in chronic urticaria (CU). In this review, we summarize the current knowledge regarding the role of platelets in chronic spontaneous and inducible urticarias. **Methods:** A literature search was performed using PubMed and Google Scholar, and the references of relevant literature were reviewed. **Results:** Overall, in CU patients, conflicting results have been obtained from the assessment of platelet indices, such as mean platelet volume, platelet count and distribution width, as well as markers of platelet aggregation and activation. Nevertheless, a few studies showed significant changes of such parameters in CU patients compared to controls, in apparent correlation with clinical severity, autoreactivity and/or inflammatory status. **Conclusions:** In the absence of de-

finite conclusions, the pathogenic role of platelets in CU needs to be further explored. Platelets might represent a link between inflammation, coagulation and histamine release in the pathophysiological network of CU.

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Definition of Chronic Urticaria

Chronic urticaria (CU) is defined as the occurrence of daily or almost daily wheals, angioedema, or both, for at least 6 weeks [1, 2]. The classification recommended by the updated international guidelines has distinguished CU into spontaneous and inducible forms [1]. Unlike physical and other inducible urticarias, in chronic spontaneous urticaria (CSU), the appearance of clinical manifestations is spontaneous and not evoked by physical-environmental stimuli.

The development of CU has been ascribed to various etiological factors, but a specific cause remains unidentified in many cases, despite repeated efforts towards an etiological search. This justifies the previous definition of 'chronic idiopathic urticaria', which is increasingly discarded in favor of the newer definition of CSU. The ter-

minology used in the past to describe this condition was heterogeneous, thus representing a potential source of confusion. In particular, in the majority of previous studies that are reviewed here, the disease was reported as CU or chronic idiopathic urticaria. For clarity purposes, considering such premises, the present article refers to the general term of CU, which replaces that of CSU in most instances, whereas inducible forms will be specified as required.

Pathophysiology of CU

Autoimmune mechanisms have been implicated in CU pathogenesis, at least in a proportion of patients, based on the presence of functional histamine-releasing IgG autoantibodies directed against IgE and/or the high-affinity IgE receptors on mast cells and basophils [3]. A screening test supportive of autoreactivity is the autologous serum skin test (ASST), the positivity of which suggests the presence of circulating histamine-releasing factors of any type, and not only of functional autoantibodies [4].

Although autoimmunity is likely to be a relevant pathomechanism in CU, it might not be the only one. Current evidence supports the possible contribution of other mechanisms, involving dysregulation of intracellular signaling pathways in basophils and mast cells or disturbed innate immunity response [5]. Recent studies displayed the histamine-releasing effect of sera from CU patients on mast cells lacking the high-affinity IgE receptor [6].

Moreover, several hints have suggested the inflammatory nature of CU [7]. A significant increase in serum C-reactive protein (CRP) concentration and plasma levels of both interleukin (IL)-6 and matrix metalloproteinase (MMP)-9 has been noted in CU patients, showing a correlation with disease activity [8–10], although Altrichter et al. [11] demonstrated that elevated levels of total and active MMP-9 in CU patients were not correlated with disease activity. However, in the study carried out by Tedeschi et al. [9], MMP-9 levels appeared to be related to disease severity and CRP levels, but not with skin reactivity to ASST or circulating histamine-releasing factors [9]. These observations seem to indicate that in CU there is an ongoing inflammatory process independent of the presence of circulating histamine-releasing factors.

Considerable interest has recently been focused on the role of activation of the coagulation system in the pathophysiology of CU. Patients with CU were found to more commonly have a positive result to the autologous plasma skin test (APST) than to ASST [12, 13]. As histamine-

releasing autoantibodies are equally present in serum and plasma, the higher APST-positive rate observed by some authors [12, 13] gave clues to the possible influence of clotting factors on the wheal-and-flare reactions induced by APST. Nevertheless, other studies [14–16] failed to confirm these results and some authors [17] pointed out that the use of APST for detecting autoreactivity in clinical practice is not superior to the use of ASST and requires further investigation.

CU patients often present elevated levels of coagulation and fibrinolysis markers, such as prothrombin fragment F1 + 2 and D-dimer [18]. In greater detail, severe exacerbations of CU are associated with a strong activation of the coagulation cascade and fibrinolysis. Plasma levels of D-dimer were found to correlate with disease severity [19–21] as well as with resistance to either antihistamines [22] or ciclosporin [23]. Plasma fibrin degradation products, D-dimer and serum CRP appear to be well correlated with each other and, in turn, significantly associated with CU severity [24], suggesting the simultaneous activation of inflammatory response and coagulation system.

The activation of coagulation in CU occurs through the involvement of eosinophils and the tissue factor (TF) pathway with thrombin generation and increased vascular permeability [25]. Proinflammatory mediators induce TF expression, the main initiator of blood coagulation, whereas activated proteases of coagulation act on protease-activated receptors (PAR) triggering inflammation. This cross-talk intensifies and maintains the activation of both systems. The thrombin generated may activate mast cells via PAR-1 and the complexes TF+FVIIa and FVa+FXa may activate these cells via PAR-2, amplifying the activation of mast cells in CU [26].

A link between inflammation and coagulation through the interaction of histamine with thrombin has therefore been hypothesized. Within the coagulation cascade, thrombin is a serine protease that also induces the activation of platelets [27]. The activation of the extrinsic coagulation pathway resulting in thrombin generation might thus support a role of platelets in CU pathophysiology.

Platelets in inflammation

Platelets play a crucial role not only in hemostasis and thrombosis, but also in many other pathophysiological processes, including vessel constriction and repair, tumor growth, infection, tissue homeostasis, inflammation and

immunity [28–31]. They express a multitude of adhesive and immune receptors on their surface, such as P-selectin, CD40 ligand (CD40L) and Toll-like receptors, and store in their granules a plethora of substances, releasing, upon activation, many inflammatory and immunomodulatory mediators, chemoattractants, growth factors and reactive oxygen species (ROS) [28, 32, 33]. Platelet activation takes place in response to various factors, such as thrombin, chemokines and microbial toxins [33]. Moreover, complement activation occurs on the platelet surface and deposition of complement also results in platelet activation [31].

Thanks to their abundant armamentarium of soluble mediators and surface molecules, platelets are considered an important tool mediating the interaction between endothelial cells and leukocytes.

Moreover, platelets have been described as a source of inflammatory mediators that are implicated in histamine release from basophils and mast cells [34–36]. Human platelets have been shown to release histamine [37, 38] and to express both high-affinity and low-affinity IgE receptors at variable levels [39, 40].

Ever-growing evidence suggests the active involvement of platelets in the pathogenesis of various inflammatory skin diseases, such as atopic dermatitis and psoriasis, through several pathomechanisms [41–43]. In some inflammatory conditions, platelet activation may represent a crucial step linking the chronic inflammatory state to the procoagulant risk [44]. This intriguing hypothesis has particularly been proposed for psoriasis as one of the possible explanations for the increased frequency of cardiovascular comorbidities [45, 46]. Significant signs of platelet activation and systemic inflammation have been reported in patients with psoriasis, especially in those with severe disease [46].

It has also been assumed that platelets may play a role in the CU-related inflammatory response. However, unlike psoriasis [47], the available data do not indicate an increased vascular risk in CU patients. At present, in fact, no association has been defined between the ongoing hypercoagulability state in CU and an increased risk of thrombosis.

Literature Search Methodology

A literature search was conducted in PubMed using the terms ‘platelet’ or ‘platelets’ and ‘urticaria’, and also in Google Scholar, considering the journal articles with the above-cited search terms in the title. There was no date

limit defined for this search, which was extended until December 2015. English-language articles addressing platelet indices and function in CU were included in the review. Additional sources were the reference lists of relevant articles, and articles shown as related citations in PubMed of the included studies. Further searches were performed in PubMed using the keyword ‘urticaria’ combined with relevant terms describing platelet-related biomarkers or inherited platelet disorders.

Platelet Indices in CU

Significance of Platelet Indices

Mean platelet volume (MPV) is the most commonly used measure of platelet size and is regarded as an *in vivo* indicator of platelet reactivity [48], as large platelets are metabolically and enzymatically more active. In fact, large platelets contain more alpha and dense granules, produce more thromboxane B₂, release more serotonin and other granule contents, and express more glycoprotein (GP) Ib [49]. Preferential aggregation of large platelets is observed after the addition of ADP (adenosine diphosphate), collagen and thrombin [49, 50].

MPV has frequently been reported as an inflammatory index in several inflammatory diseases [51–54]. While the count of platelets increases during inflammation, their volume tends to decrease or increase [55]. A number of studies showed a correlation between higher MPV values and active inflammatory disease [41, 56], although other studies have demonstrated the contrary [52, 54, 57, 58]. For instance, while small studies in patients with rheumatoid arthritis have reported a correlation between high MPV values and increased disease activity and inflammatory markers [53, 56], other studies have contradicted the above results, showing lower MPV in patients with active rheumatoid arthritis and an increase in MPV after treatment [59, 60].

Gasparyan et al. [55] provided explanations for such contradictory findings hypothesizing that high-grade inflammatory diseases result in low levels of MPV, while low-grade inflammatory diseases have the opposite effect on MPV. Nonetheless, based on these premises, the nature of MPV as an inflammatory marker remains controversial, and, thus, generalizations cannot be made. On the other hand, there are some methodological limitations in the assessment of platelet indices. In fact, these indices can be influenced not only by inflammation, but also by demographic variables, technical factors (e.g. storage time, choice of anticoagulant, type of hematology

Table 1. Summary of study results regarding the assessment of MPV in patients with CU

Ref. No.	Study details	MPV (fl) in CU patients versus controls and further comments
71	Prospective study in 40 children with CU and 40 age- and sex-matched healthy children	Significantly lower in patients (7.42 ± 0.77) versus controls (7.89 ± 0.65 ; $p = 0.004$)
72	Prospective evaluation of 34 patients with active CU and 36 healthy controls	Significantly lower in CU patients than controls (7.44 ± 0.12 vs. 7.88 ± 0.13 ; $p = 0.01$)
7	Data obtained from a large health organization in Israel, involving 12,778 patients with CU and 10,714 control subjects	Abnormally high in 28.5% of patients versus 1.2% of controls ($p < 0.0005$); values not specified
67	Retrospective assessment of 78 ASST-positive CU patients, 93 ASST-negative CU patients and 46 healthy subjects	Significantly higher in ASST-positive CU patients (9.12 ± 1.25) than in ASST-negative patients (7.95 ± 1.08 ; $p = 0.039$) and controls (7.72 ± 1.04 ; $p = 0.007$) No significant differences between ASST-negative patients and controls Significant positive correlation between CU severity score and MPV in ASST-positive patients but not in ASST-negative patients
68	Retrospective assessment of 46 CU patients resistant to AH, 245 CU patients responding to AH, and 44 sex- and age-matched healthy individuals	Higher in patients resistant to AH (10.87 ± 2.21) than in responders to AH (8.65 ± 1.74 ; $p < 0.001$) and controls (7.59 ± 1.08 ; $p < 0.001$) No significant differences between patients controlled by AH and controls
69	Cross-sectional study in 45 patients with CU, and 45 age- and gender-matched healthy controls	Significantly higher in CU patients (12.22 ± 1.24) versus controls (9.83 ± 1.54 ; $p < 0.0001$) Significantly higher in APST-positive patients (12.69 ± 0.94) versus APST-negative patients (11.51 ± 1.33 ; $p = 0.0011$) Positive correlation of MPV with platelet aggregation and USS; inverse correlation of MPV with platelet count
70	Prospective, hospital-based assessment of 194 CU patients (67 with ASST positivity) and 194 sex- and age-matched control subjects	Significantly higher in ASST-positive CU patients (12.72 ± 1.87) versus ASST-negative patients (10.32 ± 1.89 ; $p = 0.012$) and controls (10.01 ± 2.31 ; $p = 0.015$); no significant difference between the ASST-negative group and controls Positive correlation between MPV and CU severity
73	Prospective evaluation of 66 CU patients with variable disease severity and 36 sex-, BMI- (<25) and age-matched healthy subjects	No significant differences between the CU group and controls (7 in each group)

AH = Antihistamines; USS = urticaria severity score.

ogy analyzer) and several disorders, such as obesity, hypertension, smoking, hyperlipidemia, diabetes mellitus, prediabetes, atrial fibrillation, metabolic syndrome and fatty liver disease [55, 58, 61–63]. In addition, drugs and dietary components can affect platelet function. Hence, there are a huge variety of confounding factors that should be taken into account prior to the assessment of platelet indices. Consequently, it seems quite difficult and impractical to make a proper and rigorous selection of patient populations in a clinical study excluding all the potential factors which can influence platelet parameters.

As concluded by Beyan et al. [64], MPV alone is not sufficient to evaluate platelet function. Despite conflicting opinions [64], some authors supposed that the platelet distribution width (PDW), which is routinely measured by automated hematology analyzers together with MPV, can be a more specific marker [65]. However, there is as yet no ideal test for the detection of platelet activation [66].

MPV, Platelet Count and Distribution Width in CU

MPV has been investigated in a scarce number of studies in CU patients, giving rise to contradictory results. In fact, MPV was found to be either increased [7, 67–70] or

decreased [71, 72] in patients with CU (as a whole or subgroups) as compared to controls, with no difference noted in another study [73] (table 1).

In the study by Confino-Cohen et al. [7], which disclosed a significant association of CU with autoimmune disorders, a high MPV was the most common abnormal laboratory finding in patients with CU, present in 28.5%, and in 1.2% of control subjects.

Magen et al. [67] demonstrated that ASST-positive CU patients had higher mean MPV values, whereas no differences in MPV existed between ASST-negative patients and control subjects. A positive correlation of MPV with CU severity score was revealed in ASST-positive patients but not in ASST-negative patients. A recent study confirmed that MPV levels were significantly increased in ASST-positive CU subjects and were positively correlated with disease severity [70]. In another study, Magen et al. [68] observed higher CRP and MPV levels in CU patients resistant to antihistamines compared to patients who responded to antihistamine therapy, whose MPV was instead similar to that of control subjects. Notably, the group of subjects with CU refractory to antihistamines was characterized by a higher baseline clinical severity and more positive ASST results. On the basis of these results, patients with autoreactive and/or refractory CU, who generally have more severe clinical manifestations, show a tendency towards increased MPV and CRP, supporting a possible link between the involvement of platelets and the inflammatory state. According to Magen et al. [67, 68], the difference in platelet size might indicate a direct role for activated platelet mediators in CU or simply reflect bone marrow stimulation induced by the increased systemic inflammation. In another report [69], APST-positive patients were described as having a significantly higher MPV as compared to APST-negative patients, highlighting again the possible influence of autoreactivity on the results obtained.

Some of the above-mentioned studies in CU patients evaluated other platelet parameters, namely platelet count and PDW. Once more, the results were contradictory. Indeed, as compared to control patients, in CU patients the mean platelet number was reported as either increased [71] or more often unchanged [67, 68, 70, 72, 73], and PDW values were shown to be increased [69] or without substantial differences [72, 73]. An inverse correlation between MPV and platelet count was described by Chandrashekar et al. [69].

Rajappa et al. [74] found CU patients to have a decreased mean platelet count when compared to controls, with no differences observed between APST-positive and

APST-negative patients. The platelet counts did not correlate with disease severity and the level of oxidative stress or inflammatory markers.

In a study that stratified patients according to CU severity [73], there was no significant difference in platelet number between CU patients as a whole and controls, but the platelet count was significantly higher in patients with moderate-to-severe CU compared with the controls and patients with mild CU. However, all patients with CU of any severity degree showed all values of the platelet count within the normal range. Interestingly, in the same study, the platelet count correlated with serum CRP concentration, thus probably outlining a relationship with inflammatory status and disease activity.

Other Markers of Platelet Activation and Aggregation in CU

In the study by Isiksacan et al. [72], aggregation after ristocetin and thrombin receptor-activating peptide agonists was significantly decreased in CU patients versus controls, while platelet aggregation after ADP and arachidonic acid stimulation was not affected. Moreover, there were no significant differences in prothrombin time, activated partial thromboplastin time, and fibrinogen levels between patients and controls, while D-dimer levels were significantly higher in CU patients.

In patients with CU when compared to controls, and especially in APST-positive patients, Chandrashekar et al. [69] reported higher platelet aggregation and soluble P-selectin levels, which correlated positively with the urticaria severity score. Raised levels of soluble P-selectin were previously detected by Zuberbier et al. [75] in CU patients. As P-selectin is present both within endothelial cells and platelets, there has been extensive debate concerning the significance of increased levels, but the dominant current opinion is that soluble P-selectin may reflect some aspect of platelet function or activity, although definite data are lacking [69, 76, 77].

Palikhe et al. [78] recently investigated platelet-activation markers, namely P2Y₁₂ receptor and P-selectin expression on platelets together with soluble P-selectin, in aspirin-intolerant and aspirin-tolerant CU patients. In patients with CU compared with controls, the expression of P2Y₁₂ was significantly higher, with no difference in P-selectin expression. The levels were not significantly different according to symptom score and aspirin intolerance. The soluble P-selectin level was significantly higher in the aspirin-intolerant CU group.

Earlier studies in CU by Kasperska-Zajac et al. [79] failed to demonstrate platelet activation, as detected by

plasma concentrations of PF-4 and/or β -thromboglobulin. In another study performed by the same authors [80], the circulating level of the platelet-derived chemokine PF-4 was not increased in CU patients, regardless of either the occurrence of euthyroid Hashimoto's thyroiditis or the response to ASST.

In contrast, Katayama et al. [81] more recently observed elevated concentrations of PF-4 and β -thromboglobulin in CU patients in conjunction with a high concentration of D-dimer and prothrombin fragment F1 + 2. These platelet-derived and coagulation cascade factors returned to normal levels after the remission of urticaria following treatment with antihistamines. In some cases, signs of platelet activation were detected again in the event of urticaria recurrence.

Platelets release ROS upon activation and these ROS might influence the activation and degranulation of basophils and mast cells [82, 83]. One study has suggested the existence of significant systemic inflammation and platelet oxidative stress in patients with CU [74]. The authors assessed markers of platelet oxidative stress (malondialdehyde, superoxide dismutase and glutathione peroxidase). In patients with CU, platelet superoxide dismutase and glutathione peroxidase were significantly lowered, while platelet malondialdehyde levels were significantly elevated in comparison to healthy controls. IL-6 and CRP were also significantly elevated and correlated with platelet oxidative stress parameters. Interestingly, all inflammatory and oxidative stress parameters showed a significant correlation with the urticaria activity score. In a small study performed in 10 patients with CU and 10 healthy controls [83], CU was found to be significantly associated with enhanced ROS production in platelets.

Among the immunoregulatory proteins contained in platelets, there is CD40L, a transmembrane molecule that is expressed by a variety of cells, but mainly by activated T-lymphocytes and platelets [30]. CD40L may be cleaved into a soluble form (sCD40L) that has a cytokine-like activity. Agonists, including thrombin, stimulate CD40L expression on platelets and the release of sCD40L. Long-term platelet activation leads to complete conversion of CD40L to sCD40L. Circulating sCD40L is believed to derive predominantly from activated platelets and may therefore reflect platelet activation [28]. The platelet CD40L-CD40 axis is believed to serve as a central link between endothelium/coagulation and inflammation. Upon exposure to CD40-expressing vascular cells, platelet-derived sCD40L can induce the expression of adhesion molecules, such as P-selectin, and initiate the release of TF

and IL-6 [28, 84]. In addition, sCD40L can enhance ROS release from platelets and platelet aggregation and activation [32, 85]. It has been demonstrated that serum sCD40L concentration is significantly increased in CU patients as compared to healthy subjects [86, 87]. In contrast, sCD40L concentration was unaltered in platelet-poor plasma [88]. This indicates that the measurement in the serum may be more appropriate to examine the total pool of sCD40L stored in platelets and other cells.

Platelets in Chronic Inducible Urticaria

Only very scarce data exist on the potential involvement of platelets in inducible forms of CU. Studies with a small sample size evaluated the role of platelet activation in cold urticaria and provided controversial results [89–91]. A significant increase in soluble P-selectin was seen in patients with dermatographic urticaria when compared to healthy controls and rhinitis subjects [75].

The platelet-derived chemokines PF-4 and β -thromboglobulin displayed elevated serum levels in patients with delayed pressure urticaria [92]. Furthermore, the plasma sCD40L concentration was significantly increased in these patients compared to the healthy controls [93]. Therefore, delayed pressure urticaria seems to be associated with an increased systemic release of sCD40L, which is believed to derive predominantly from activated platelets.

Familiar cases of aquagenic urticaria and Bernard-Soulier syndrome have been described [94]. Bernard-Soulier syndrome is a rare bleeding disorder, mostly inherited in an autosomal-recessive pattern, characterized by defects of the GPIb-IX-V complex, a platelet receptor for von Willebrand factor, thrombocytopenia, giant platelets and the absence of ristocetin-induced platelet aggregation.

Conclusions

The role of platelets in CU has been investigated by a limited number of studies so far, and the available data appear to be equivocal. However, in the absence of definite conclusions, some findings seem to suggest the involvement of platelet activation in patients with CU, in connection with the systemic inflammatory response. Activated platelets might have effects on histamine-releasing effector cells, probably through activation of the coagulation cascade.

Cumulative current evidence indicates that the simple determination of platelet indices is not reliable and lacks useful clinical implications. Conflicting results regarding the assessment of MPV and other platelet indices in CU patients might also be due to the difficulties in excluding the multitude of confounding factors, as mentioned above. Most of the studies on this topic were small and retrospective, and the retrospective nature of some studies could have compromised the correct evaluation of the numerous exclusion criteria.

Furthermore, the changes in platelet indices that were sometimes detected in forms with greater severity, auto-

reactivity and/or high-grade inflammation can lead to the supposition that the contradictory data on platelet indices in CU can also be linked to the heterogeneity of the patients enrolled in the diverse studies, who might have had different characteristics in terms of severity, autoreactive nature and/or the intensity of the inflammatory status. Unfortunately, only very few studies distinguished CU patients on the basis of such characteristics. The pathogenic role of platelets in CU deserves further attention and investigation, and more studies are needed to assess the relationship between platelet activation and CU activity/severity.

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