

ORIGINAL ARTICLE

Plasma IL-6 concentration correlates with clinical disease activity and serum C-reactive protein concentration in chronic urticaria patients

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Summary

Background Our previous study was the first to demonstrate enhanced plasma IL-6 concentrations in chronic urticaria (CU). It is known that C-reactive protein (CRP) is a sensitive marker of an underlying systemic inflammation, triggered mainly as a response to IL-6.

Objective To evaluate plasma IL-6 concentration in CU patients relating to the clinical disease activity and serum CRP concentration.

Methods Serum CRP and plasma IL-6 concentrations were measured in 58 CU patients and 30 healthy subjects. Ten CU patients were evaluated twice, during the active period as well as upon the spontaneous clinical remission of the disease. CU activity was assessed with the use of the symptom scores recommended by EAACI/GALEN/EDF guidelines.

Results IL-6 and CRP concentrations were significantly increased in CU patients as compared with the healthy subjects, whereas they decreased remarkably upon the spontaneous remission. IL-6 concentration was associated with weekly urticaria activity scores and also significant differences were found between patients showing different degrees of urticarial activity. Significant correlation was observed between IL-6 and CRP concentrations.

Conclusions and Clinical Relevance This study reinforces evidence that, apart from a local cutaneous inflammation, CU is associated with a systemic inflammatory response. Such acute-phase response is manifested by increased circulating IL-6, which varies along with CRP changes and may be related to the urticarial activity.

Keywords acute-phase response, chronic urticaria, C-reactive protein (CRP), IL-6, systemic inflammation

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Introduction

Chronic urticaria (CU) is associated with mast cell activation accompanied by inflammatory response, which may be manifested by increased serum concentration of C-reactive protein (CRP) [1–3]. CRP is a sensitive systemic marker of inflammation and tissue damage, produced mainly in the liver, responding to IL-6. IL-6 is believed to be the central regulator of the immunological and inflammatory processes and in humans the cytokine is elevated in many inflammatory diseases [4–7].

So far, only scarce research has associated the systemic inflammatory response with CU. Our previous minor study suggested that plasma IL-6 concentration is significantly increased in CU patients; however, the patients were not

divided according to the degree of their urticarial activity [8]. To gain some better insight into the significance of IL-6, it would be interesting to know whether plasma IL-6 concentration is associated with any clinical activity as well as serum CRP concentration in CU. Moreover, only some studies were devoted to the outcome of blood quantitative or qualitative abnormalities upon inactive CU. Therefore, we have designed another part of the study to verify whether CU patients in complete clinical remission, receiving no treatment, continue to show increased plasma IL-6 concentration. Taken together, we measured plasma IL-6 concentration in CU patients in the active stage and upon clinical remission to evaluate then any possible association with serum CRP concentration as well as clinical activity scores.

Methods and materials

Subjects

Fifty-eight patients (15 men and 43 women; median age 40 years) with CU were selected for the study. Ten patients had concomitant dermatographism. In all cases, any known causes of CU were ruled out by appropriate investigations. Each patient underwent the following routine laboratory tests: full blood count, urine analysis, erythrocyte sedimentation rate, CRP, serum glucose, hepatic functions, and creatinine examination. In addition, stool examination for parasites, hepatitis serology (anti-HCV, HBsAg), rheumatoid factor, antistreptolysin-O, Igs, complement, antinuclear and antithyroid microsomal antibodies, thyroid function tests (TSH and free thyroxine-fT4), chest X-ray, and abdominal ultrasonography were performed.

The *Helicobacter pylori* infection was excluded upon the negative results of *H. pylori* antigen stool test and/or rapid urease test performed during gastrofiberscopy. Gastrofiberscopy was performed in patients showing positive gastric symptoms and/or a positive result of *H. pylori* antigen stool test. Our centre does not perform routine-specific IgG and IgM antibodies evaluation for *H. pylori* in serum (ELISA test); the reasons are limitations of the method itself as well as lack of our own relevant experience. Three patients had a positive result of the test (performed in another clinic) as well as concurrent gastric ailments, while gastrofiberscopy proved no *H. pylori*.

All the patients showed active urticaria at the time of assessment and were divided into subgroups according to their urticarial activity as well as autologous serum skin test (ASST) response.

It should be mentioned that our patients underwent a panel of extensive tests that are normally not recommended as routine screening for patients with CU. However, taking into consideration a specific objective of our study, such a strong selection of CU patients was demanded in order to eliminate some processes, which not only condition the occurrence of CU but may also effect in elevated concentration of inflammatory mediators. This could then obstruct proper interpretation of the results.

Assessment of urticaria activity scores

Urticaria activity score (UAS) was calculated as recommended by EAACI/GA2LEN/EDF guidelines [9] and as earlier described by Maurer group [10]. Weekly UAS was estimated according to the number of weals and pruritus intensity, which had appeared during a week before and on the day of blood sampling, applying the following scheme: no weals = 0, 1–10 weals = 1, 11–50 weals = 2, > 50 weals = 3 and pruritus intensity: no = 0, mild = 1, moderate = 2, severe = 3. UAS scores: daily (minimum = 0; maximum = 6) and weekly by adding the daily score

values (minimum = 0; maximum = 42). Consequently, weekly UAS were graded as follows: 0–14 (mild), 15–29 (moderate), and 30–42 (severe).

Our study comprised 25 patients with mild CU, 19 patients with moderate CU, and 14 patients with severe urticaria symptoms as per the weekly UAS. Patients with severe CU represented a group that before the test showed highly intensified symptoms, only hardly controlled by overstandard doses of antihistamines (20 mg of levocetirizine or desloratadine).

In addition, 10 out of 58 CU patients were classified for the second stage of the study performed upon the clinical remission. Remission was defined as the absence of any urticaria symptoms without medication applied during at least two consecutive months.

H1 and H2 antihistamine drugs were withdrawn at least 10 days before blood sampling. No patient had been taking corticosteroids or immunosuppressant drugs for at least 6 weeks.

None of the patients had associated chronic inflammatory disorders or a history of recent acute infectious disease (1 month before) or any other factors, which could have influenced the marking scores.

Thirty sex-matched, age-matched, and BMI-matched healthy subjects formed the control group.

All the subjects submitted respective written consent and the study was approved by the University Committee of Ethics.

Blood collection

All blood samples were obtained between 7:00 and 9:00 hours by antecubital puncture. EDTA was used as an anticoagulant.

Assay of interleukin-6

Plasma IL-6 concentration was determined by ELISA (R&D Systems Inc., Minneapolis, MN, USA), according to the manufacturers' protocol. The intraassay and interassay CV were 4.2% and 3.3% for IL-6. Sensitivity of the assay was 0.70 pg/mL.

Assay of C-reactive protein

Serum CRP concentration was measured by the turbidimetric latex agglutination method (CRP-Latex, BioSystems SA, Barcelona, Spain) with a detection limit of 1.0 mg/L. Elevated serum CRP was defined as higher than 5.0 mg/L.

Autologous serum skin test

Intradermal ASST was performed following the method by Sabroe et al. [11]. Serum-induced weal of diameter greater by at least 1.5 mm than that of a control weal

induced by physiological saline was accepted as positive. Skin prick test with 10 mg/mL histamine served as a positive control.

Statistical analysis

Results are expressed as median and interquartile ranges. Because data were not distributed normally, non-parametric tests were used. The Kruskal–Wallis variance analysis was used for screening differences between the groups. The Mann–Whitney *U*-test was used to compare data between the patient groups and the healthy controls. The Wilcoxon's paired test was used to evaluate differences within the same group. Spearman's rank test was used for correlations. A probability value of $P < 0.05$ was assumed to be significant.

Results

Plasma interleukin-6 concentration and correlation with urticaria activity score

Plasma concentration of IL-6 was significantly higher in CU patients as compared with the healthy subjects (median: 1.85 vs. 1.1 pg/mL; $P < 0.001$) (Fig. 1). There were significant differences in plasma IL-6 concentration between the CU patients with mild, moderate, and severe symptoms (median: 1.2 vs. 1.9 vs. 3.3 pg/mL, respectively; $P < 0.05$ and < 0.001) (Fig. 2). Concentration of this cytokine in mild CU group did not differ significantly as compared with the healthy subject (Fig. 2).

Plasma IL-6 concentration showed significant correlation with the disease activity as assessed by calculating the cumulative UAS during 7 days before blood sampling ($r = 0.638$, $P < 0.0001$) (Fig. 3).

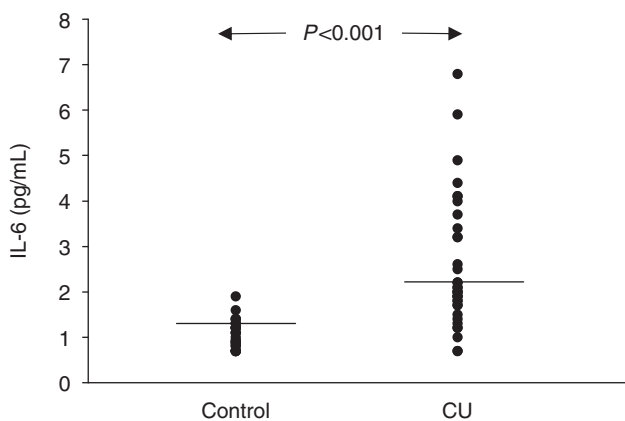


Fig. 1. Individual plasma IL-6 concentration in healthy subjects and chronic urticaria (CU) patients. Horizontal lines represent median value.

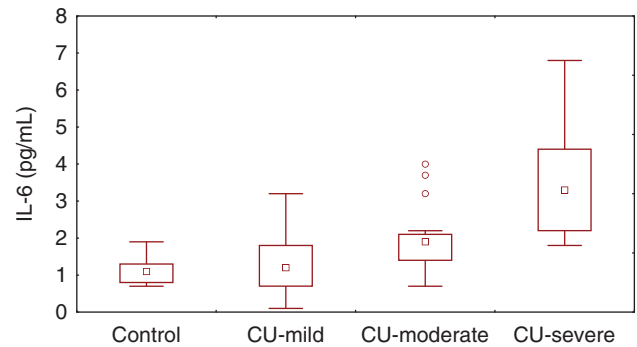


Fig. 2. Plasma IL-6 concentration in healthy subjects and chronic urticaria (CU) patients with different disease activity. CU-mild vs. CU-moderate vs. CU-severe, $P < 0.05$ and < 0.001 , respectively; control vs. CU-moderate vs. CU-severe $P < 0.001$; control vs. CU-mild $P > 0.05$.

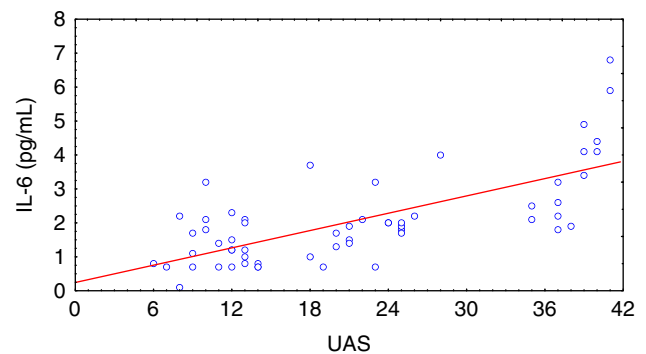


Fig. 3. Correlation between plasma IL-6 concentration and weekly urticaria activity scores (UAS) $r = 0.638$, $P < 0.0001$.

No significant differences in the cytokine concentration were observed between ASST(+) and ASST(–) CU patients (median: 1.8 vs. 1.98 pg/mL; $P > 0.05$).

In the statistical analysis, IL-6 concentration below the detection limit (0.7 pg/mL) was assumed to show such a value.

Serum C-reactive protein concentration and correlation with urticaria activity score

Serum CRP concentration was significantly higher in CU patients as compared with the healthy subjects (median: 3.85 vs. 1.32 mg/L; $P < 0.001$) (Fig. 4). In addition, there were significant differences in serum CRP concentration between the CU patients with mild, moderate, and severe symptoms (median: 2.1 vs. 4.6 vs. 7.7 mg/L, respectively; $P < 0.05$ and < 0.001) (Fig. 5). In contrast to CU patients with moderate or severe symptoms, serum CRP concentration in mild CU group did not differ significantly as compared with the control group (Fig. 5).

Serum CRP concentration was significantly correlated with the disease activity as assessed by weekly UAS ($r = 0.714$, $P < 0.0001$).

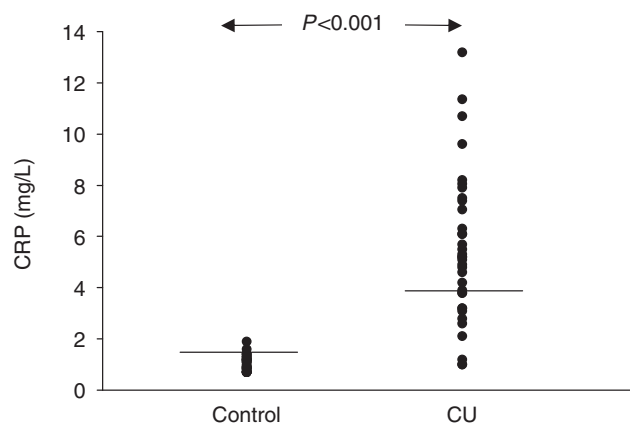


Fig. 4. Individual serum CRP concentration in healthy subjects and chronic urticaria (CU) patients. Horizontal lines represent median values.

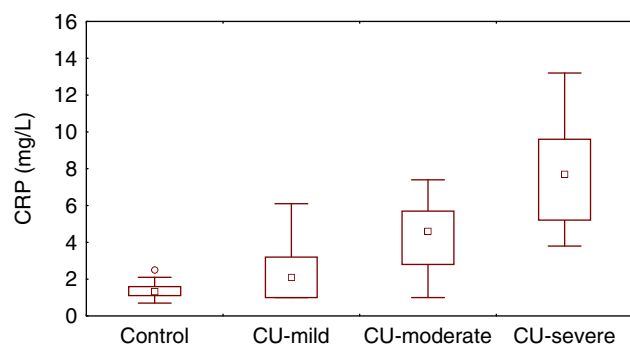


Fig. 5. Serum CRP concentration in healthy subjects and CU patients with different disease activity. CU-mild vs. CU-moderate vs. CU-severe $P < 0.001$; control vs. CU-moderate vs. CU-severe $P < 0.001$; control vs. CU-mild $P > 0.05$.

No significant differences in CRP concentration were observed between ASST(+) and ASST(−) CU patients (median: 3.95 vs. 4.07 mg/L; $P > 0.05$).

In the statistical analysis, CRP concentration below the detection limit (< 1 mg/L) was assumed to show such a value.

Analysis of serum C-reactive protein concentration in chronic urticaria patients, considering the disease activity and normal laboratory values

Along with the increasing CU activity (from mild to severe), a greater share of patients showed the CRP values above the upper limit of the normal laboratory range (> 5 mg/L) (Table 1). Most of the CRP values exceeding the normal range upper limit were observed in severe CU (78.6% patients); however, this was not the case in all patients. Three patients of this group (21.4%) showed the CRP value within the normal laboratory range. Furthermore, in two out of 25 (8%) of the mild CU group and in

Table 1. Analysis of serum C-reactive protein (CRP) concentration in chronic urticaria (CU) patients

CRP concentration	Mild CU ($n = 25$)	Moderate CU ($n = 19$)	Severe CU ($n = 14$)
Below the detection limit (< 1 mg/L)	9/25 (36%)	2/19 (10.5%)	0/14 (0%)
Beyond upper limit of the normal laboratory range (> 5 mg/L)	2/25 (8%)	8/19 (42%)	11/14 (78.6%)

n , number of patients.

eight out of 19 (42%) of the moderate CU patients, the CRP values were confirmed beyond the upper limit.

In nine out of 25 (36%) of the mild CU patients, the CRP values were below the detection limit (< 1 mg/mL), which was not the case in any of the patients with severe disease activity (Table 1).

Correlation between plasma interleukin-6 and serum C-reactive protein concentrations

Significant correlation was noted between circulating concentration of IL-6 and CRP ($r = 0.859$, $P < 0.0001$) in CU patients.

Changes in plasma interleukin-6 and serum C-reactive protein concentration during the active phase of the disease and upon remission

Plasma IL-6 and CRP concentrations in CU patients upon remission were significantly lower as compared with active CU ($P < 0.05$) and did not differ significantly from the healthy subjects. Individual changes in plasma IL-6 concentration in CU patients during the active phase of the disease, compared with the remission phase are shown in Fig. 6.

Discussion

Very little is known about the role of IL-6 in urticaria. Until now, increased local activity of IL-6 has been observed in the skin of patients with delayed pressure urticaria [12] and chronic idiopathic urticaria [13]. In addition, elevated circulating IL-6 has been found in acute urticaria [14].

In the present study, plasma IL-6 concentration was significantly higher in CU patients as compared with the healthy controls. There was a significant correlation between IL-6 concentration and the clinical activity score of urticaria (weekly UAS) and also some significant differences were found between patients showing different degrees of urticaria activity (mild, moderate, and severe) according to weekly UAS. Such association with the urticarial activity scores indicates a potential role of IL-6 as a marker of the urticarial activity. Nevertheless, an

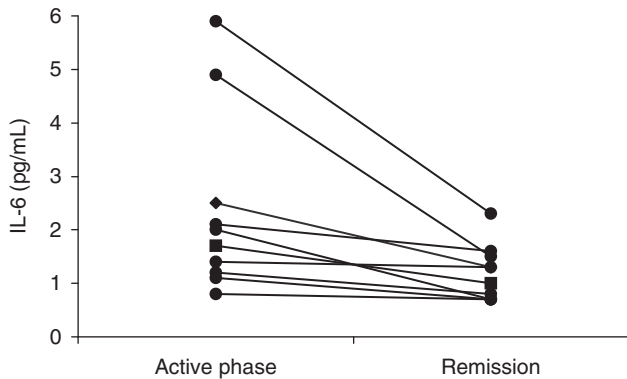


Fig. 6. Changes in plasma IL-6 concentration in chronic urticaria (CU) patients during the active phase in comparison with the remission phase of the disease (significant decrease of IL-6; $P < 0.05$).

extensive study should be performed to determine a normal laboratory range for IL-6 in a given population.

No significant differences were found between ASST-positive and ASST-negative CU patients. A negative ASST has a strong negative predictive value for the presence of circulating histamine-releasing autoantibodies, whereas positive ASST indicates the autoimmune/autoreactive origin of CU [15]. Therefore, the observation of raised IL-6 concentrations independent of ASST positivity suggests that in CU an elevated IL-6 plasma concentration reflects the inflammatory process independent of the involvement of autoimmune/autoreactive phenomena.

Significantly lower plasma IL-6 concentration in CU patients during spontaneous remission as compared with the active phase of the disease has been observed. In addition, respective values did not differ significantly from those of the healthy subjects. Therefore, we suggest that elevated IL-6 concentration characterizes the active phase of CU as much as the cytokine behaving dynamically during the clinical course of the disease.

Interestingly, IL-6 directly and CRP by activating the complement pathway may induce the expression of the tissue factor [16, 17]. It is known that the tissue factor pathway of coagulation/fibrinolysis cascade is activated in CU, which parallels the disease activity [1, 3, 18, 19]. Taking this into account, the relationship between the coagulation/fibrinolysis markers and IL-6 concentration should be investigated.

The source of IL-6 in CU has not been identified. Mast cells and other inflammatory cells types identified in skin biopsies of CU patients, including monocytes, basophils, eosinophils, activated T cells, and neutrophils release this cytokine [4, 7, 13, 16]. Thus, elevated IL-6 is likely to result from the activation of different inflammatory cells involved in urticarial processes, especially the mast cells. So far, it has been suggested that the increased number of tryptase-positive, chymase-negative mast cells in the

lesioned skin of CU may be an important source of IL-6 and IL-5 [13].

We also do not know whether IL-6 is released from the site of the urticarial inflammation into the bloodstream and/or released by the circulating inflammatory cells. Interestingly, up-regulation of IL-6 mRNA in peripheral blood mononuclear cells of patients with acute urticaria has been demonstrated [20].

In our study, the serum concentration of CRP was significantly increased in CU patients, which is consistent with results of the previous studies [8]. In addition, significant correlation was observed between IL-6 and CRP concentrations in the CU patients. Studies of a variety of diseases have consistently pointed to the correlation between the circulating IL-6 and CRP. However, elevated IL-6 concentrations were not always accompanied by the increased CRP, for e.g. in lupus [4]. In our previous study, we did not find any significant correlation between IL-6 and CRP in CU; however, the study was limited by a small number of subjects [8].

Interestingly, some correlation was found between CRP and matrix metalloproteinase-9 (MMP-9) concentrations in active period of the disease [3, 10]. Therefore, it should be asked whether there is any relationship between the concentration of circulating MMP-9 and IL-6 in CU.

Similar to IL-6 behaviour, the CU patients have raised serum CRP concentration correlating with the weekly UAS and there were significant differences between the CU patients with mild, moderate, or severe symptoms, but not between mild CU and the control group. None of the patients with severe urticaria showed CRP scores below the detection limit. On the other hand, in three out of 14 patients with severe CU, CRP concentrations did not exceed the normal value. Thus, despite severe disease activity, CRP concentration can remain within the normal laboratory range. In addition, two out of 25 patients with mild CU had CRP values above the upper limit of the normal laboratory range. Such observations may restrict the clinical usefulness as a biomarker for the disease activity in individual patients. However, even in patients showing the values within the normal range during the symptomatic period, they decreased below the detection limit upon remission. It should be emphasized that there are some individual differences in acute-phase inflammatory marker responses to different stimuli. This was confirmed by our study where at the same UAS degree, very high individual differences were observed in CRP and IL-6 values.

Taken together, more severe CU in most cases is characterized by systemic inflammatory process, indicated by the increased circulating CRP and accompanied by parallel changes in IL-6 concentration. Such a pattern is compared with other chronic inflammatory disorders. The CU patients may then require a more thorough evaluation and more extensive therapy, including corticosteroids or immunosuppressants.

Assuming also that CRP is a very powerful predictor of future cardiovascular events in healthy adults, even a slight elevation in the CRP baseline concentration is enough to increase significantly the risk of a cardiovascular disease [21, 22]. We should test whether CU patients who show stronger inflammatory response are more vulnerable to possible future cardiovascular disease or dysregulation of the existing disorders. It seems especially important in long-lasting severe CU disease.

In conclusion, the study has been the first one to show that increased plasma IL-6 concentration is a transient and reversible phenomenon accompanying CU, occurring during the active period, withdrawn upon remission of the disease, and correlated with the urticarial activity scores. Moreover, the observed association between plasma IL-6 and serum CRP concentrations indicates that elevated IL-6 is reflected in increased CRP concentration.

Clinical relevance

The study brings stronger evidence to prove that apart from a local inflammation, CU is associated with a systemic inflammatory response. Such acute-phase response is manifested by increased circulating IL-6, which varies along with CRP changes and may be related to the urticarial activity.

Whether the circulating IL-6 contribute to the pathogenesis of CU or is merely a secondary consequence, calls for future investigation. It would be interesting to determine whether blocking of IL-6 action in severe CU may have any beneficial effect.

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