Lithuanian University of Health Sciences

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Serum C-reactive protein concentration in chronic urticaria patients

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for the degree of Master of Medicine

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SUMMARY

Master thesis by Alisa Usher.

Title: Serum C-reactive protein concentration in chronic urticaria patients.

The aim of the theses: To investigate serum C- reactive protein level in patients with chronic spontaneous urticaria.

Objectives of the study: 1. To identify inflammatory markers in peripheral blood (C-reactive protein concentration, leucocyte, lymphocyte, eosinophil count) in patients with chronic spontaneous urticaria. 2. To evaluate the presence of angioedema and serum C-reactive protein levels according to the activity of chronic spontaneous urticaria. 3. To determine C-reactive protein levels in chronic spontaneous urticaria patients with positive autologous serum skin test**.** 4. To evaluate peripheral blood autoimmunity markers (anti-TPO and ANA) according to the activity of chronic spontaneous urticaria.

Methodology: This study is a retrospective analysis of medical records and laboratory test results of 48 patients with chronic spontaneous urticaria. All of them were consulted during 2015-2016 in department of Immunology and Allergology at the hospital of LSMU Kaunas clinics. According to the UAS7 patients were divided (urticaria activity score 7), patients with chronic spontaneous urticaria were divided into 3 groups: mild urticaria (1-15), moderate urticaria (16-27) and severe urticaria (28-42); according autologous serum skin test results patients with chronic spontaneous urticaria were divided into 2 groups: autologous serum skin test positive and negative group, CRP levels, leucocyte, lymphocyte, eosinophil count were measured in PB. Statistical analysis was performed using SPSS statistics for Windows v. 20.0.

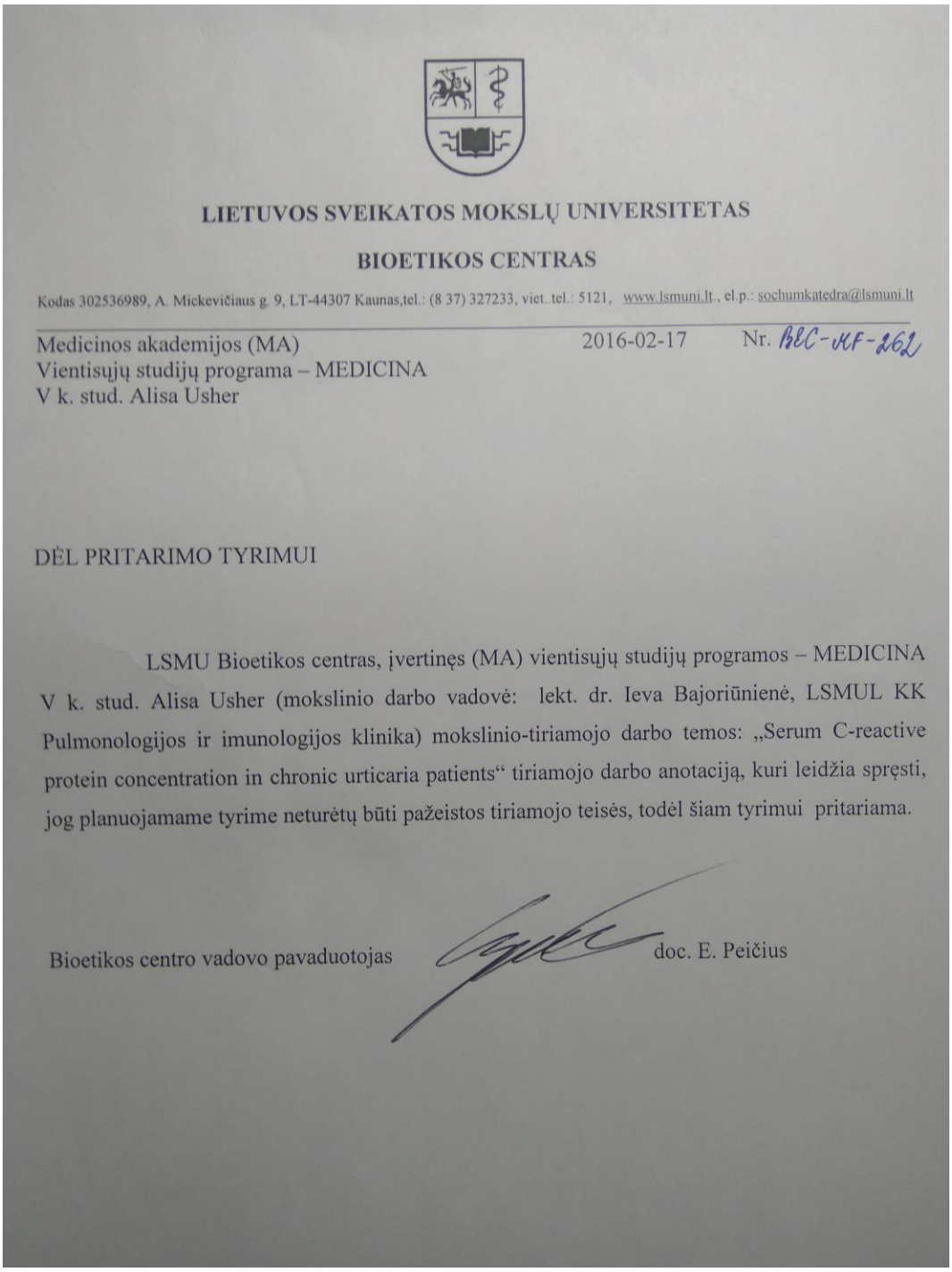
Results: The patients with severe chronic spontaneous urticaria had higher C- reactive protein levels compared with the patients suffering from mild and moderate chronic spontaneous urticaria (3.6 (1.8) vs 0.6 (0.5) and 0.7 (0.8) mg/L, $P< 0.05$). 9 patients (30%) with positive autologous serum skin test had higher urticaria activity score 7, compared with 21 patients (70%) with negative autologous serum skin test (38.1(7.2) vs 20.0(10.4), $P< 0.05$). There were no significant differences in peripheral blood neutrophil, lymphocyte, eosinophil and IgE and the C-reactive protein levels in chronic spontaneous urticaria patients with positive and negative autologous serum skin test.

Conclusions: 1. chronic inflammation are common in patients with chronic spontaneous urticaria, the prevalence of patients with increased serum CRP levels is 20.8 %. 2. Serum C- reactive protein levels were significantly higher in patients with severe chronic spontaneous urticaria, but no significance was found with the presence of angioedema 3. There were no differences in C- reactive protein levels in chronic spontaneous urticaria patients with positive and negative autologous serum skin test. 4. No difference was found between immunity markers (anti-TPO and ANA) to disease activity.

CONFLICT OF INTEREST

The author reports no conflicts of interest.

ETHICS COMMITTEE CLEARANCE



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DĖL PRITARIMO TYRIMUI

LSMU Bioetikos centras, įvertinęs (MA) vientisųjų studijų programos – MEDICINA V k. stud. Alisa Usher (mokslinio darbo vadovė: lekt. dr. Ieva Bajoriūnienė, LSMUL KK Pulmonologijos ir imunologijos klinika) mokslinio-tiriamojo darbo temos: „Serum C-reactive protein concentration in chronic urticaria patients“ tiriamojo darbo anotaciją, kuri leidžia spręsti, jog planuojamame tyrime neturėtų būti pažeistos tiriamojo teisės, todėl šiam tyrimui pritariama.

Bioetikos centro vadovo pavaduotojas doc. E. Peičius

LIST OF ABBREVIATIONS

ANA - anti nuclear antibody

APR - acute phase response

ASST - autologous serum skin test

AU - autoimmune urticaria

CAU - chronic autoimmune urticaria

CIU - chronic idiopathic urticaria

CRP - C-reactive protein

CSU - chronic spontaneous urticaria

CU - chronic urticaria

RA - rheumatoid arthritis

SLE - systemic lupus erythematous

TPO - thyroid peroxidase antibodies

UAS7 - urticaria activity score 7

NSAIDs - nonsteroidal anti-inflammatory drugs

ACE inhibitors - angiotensin-converting-enzyme inhibitors

INTRODUCTION

Urticaria is a recurrent disease that clinically affects the skin by appearance of highly pruritic erythematous lesions. Chronic urticaria (CU) is present on most days of the weeks, appearing for 6 weeks or longer. These lesions develop from vasodilatation and edema of the dermis and can be featured alone or together with angioedema (localized deeper swelling of mucosa or skin) [1].

According to the GA2LEN/EAACI/WAO/EDF guidelines urticaria is largely known as chronic idiopathic urticaria (CIU) (or spontaneous urticaria) [2]. Yet different sources also divided urticaria into- CIU and chronic autoimmune urticaria (CAU) [3]. Etiology is largely unknown but in some cases when causative factors are identified as autoimmunity, autoimmune thyroid disease is the most common. But sources have shown that cross link between several autoimmune diseases have even bigger effect on disease activity [21]. To identify severity class of urticaria, urticaria activity score system (UAS7) is being used [4].

Urticaria has been questioned in relation to several factors to be able to connect some laboratory markers to disease severity. Among them are C reactive protein (CRP), autoimmune biomarkers like ANA, anti-TPO, IgE and autologous serum skin test (ASST).

The major physiological development secondary to tissue damage and inflammation results in an increase in concentrations of specific reactants causing acute phase response. The purpose of this study was to be able to associate reactants like CRP to disease activity and other blood markers to its elevation or to the activity alone. The goal is to be able to understand the physiological changes in the body during flaring episodes to help in diagnosis or understand pathophysiology of the disease.

THE AIM AND OBJECTIVES

The Aim of the thesis was to investigate serum C- reactive protein levels in patients with chronic spontaneous urticaria.

Objectives of the study:

1. To identify inflammatory markers in peripheral blood (C-reactive protein concentration, leucocyte, lymphocyte, eosinophil count) in patients with chronic spontaneous urticaria.
2. To evaluate the presence of angioedema and serum C-reactive protein levels according to the activity of chronic spontaneous urticaria.
3. To determine C-reactive protein levels in chronic spontaneous urticaria patients with positive autologous serum skin test**.**
4. To evaluate peripheral blood autoimmunity markers (anti-TPO and ANA) according to the activity of chronic spontaneous urticaria.

LITERATURE REVIEW

Urticaria, also named hives (sometimes called wheals), is a recurrent disease of highly pruritic erythematous lesions (plaques). Chronic urticaria (CU) is present on most days of the weeks, appearing for 6 weeks or longer. It can be featured alone or together with angioedema (localized deeper swelling of mucosa or skin) and according to sources is present in 40% of patients with CU [1].

There can be many reason for the appearance of wheals but as appose to acute urticaria, CU etiology is much less identified. In 80-90% of cases the contributing factor is not established, but several pathogenetic mechanisms try to explain the formation of wheals.

It is proposed that mast cell degranulation and discharge of histamine are predominantly responsible for the breakout of wheals but in more than 50% of cases no allergic episode is found to explain the release of histamine. In addition there are possible overlapping mechanisms, for example physical urticaria that may manifest as acute and as chronic. For this reason according to the GA2LEN/EAACI/WAO/EDF guidelines, classification of chronic urticaria is now largely known as chronic idiopathic urticaria (CIU) (or can be called spontaneous urticaria- CSU) [2].

Another theory for the disorder suggests autoimmune association, the view came from the realization that thyroid dysfunction and thyroid autoantibodies are more common in patients with CSU.

This lead to the search for autoantibodies and other immune biomarkers that could be responsible for increased release of histamine. And several different researches suggest that most patient with chronic urticaria are divided into 55% with CIU and 45% with chronic autoimmune urticaria (CAU) [3].

Activity of chronic urticaria

Assessment of chronic urticaria activity depends on the patient's documentation of their symptoms. It is based on the severity of the itch and number of wheals.

The latest EAACI/GA2 LEN/EDF guidelines recommend using a symptom score, the urticaria activity score (UAS, UAS7).

Daily UAS values can range from: 0 to 6 points, given the number of wheals (0–3 points) and the intensity of pruritus (0–3 points) present on that day. After 7 days of documentation the daily average is summed up over one week for maximum score of 42. Higher score represents more severe activity of the disease.

It is divided into: mild urticaria (UAS7 = 7–15), moderate urticaria (UAS7 = 16–27) and severe urticaria (UAS7 = 28–42) [4, 5].

Serum C- reactive protein levels in patients with chronic urticaria

Being an inflammatory disease, CSU is characterized by acute phase response (APR).

C-reactive protein (CRP) is a marker of systemic activity, expressing the systemic effects of inflammatory mediators related with CSU.

The connection of inflammatory markers, especially CRP, with the severity and evolution of the disorder indicates that the marker can be used as a diagnostic tool. Investigating it can also help to establish a basis for pathogenesis, but it is not yet known if the marker represents the pathogenesis or is a result of the disorder. Some studies have shown that increased release of CRP concentration correlates with disease severity; therefore characterization of APR in CSU can result in better understanding of disease activity [6]. But CRP is not exclusive to inflammatory disorders and it is a sensitive marker in infections also. It is possible that when measuring CRP concentration in urticaria episodes the patients have concomitant inflammatory disorder or infections, therefore other markers of infection such as leukocytes, neutrophils and elevated ESR need to be tested.

In a study by R Lin *et al*, all these parameters were tested, and he found that most of the patients with CU did have elevated CRP levels and only one patient was found with elevated inflammatory markers other than CRP. His urticaria was attributed to an infection [7].

Even though it is a sensitive marker, in different inflammatory disorders CRP level varies, it could be increased or not be increased at all, the reason is not known, and in CU patients according to different researches the levels of CRP are diverse between the patients.

Kasperska-Zajac *et al* found in their search that few patients with severe activity of CSU demonstrated no CRP increase above the normal level, moreover other patients with mild activity of CSU did displayed CRP concentration above the normal [8].

On the other hand, Takahagi *et al* found in their research that patients with severe CSU had elevated CRP concentration more than patients with moderate or mild disease activity. He suggested that CRP levels may be useful for the assessment of disease activity and can help in understanding its part in the pathogenesis [9].

Currently many studies explore the relation of CRP with activity and severity of CSU, and many do find connection between increased or normal values of CRP and the disease, but most suggest that more inflammatory markers of acute phase response can be useful, such as procalcitonin or IL-6 and their

association with concentration of CRP that can indicate on systemic inflammatory response in CSU, and be involved in the pathogenesis of the disease [10].

Autoimmune urticaria

Because etiology of CSU is largely unknown and the cause is identified in only small number of patients, it is suggested that autoimmune mechanism is involved in the pathogenesis.

It is perceived that about 40% of the patients with autoimmune chronic urticaria have histamine-releasing autoantibodies against IgE receptor and up to 10% have specificity against IgE itself [11].

The presence of autoantibodies is hypothesized to be related with the activity of the disease.

The autologous serum skin test (ASST) is the most generally used method to differentiate between CSU patients and those with CAU. It is used as a screening test to determine functional histamine release from basophils or mast cells, where positive results are in patients who are found with the autoantibodies, and they are considered to have higher chances to develop urticaria due to endogenous factors compared to patients with negative ASST.

Boguniewicz *et al* described the patients with positive ASST as having more severe and difficult-to-control urticarial with persistent attacks (>5 times/week) [12].

The ASST involves intradermal injection of patients own serum. A positive test is represented as a serum-induced weal response with a diameter of > 1.5 mm [13] (figure 1).

But although the ASST is an effective means for screening patients with circulating endogenous factors for wheal formation, its specificity for the presence of anti-FcεRI is not very good, at best it is 70%- 80% sensitive and specific. Improved screening tests are being sought [14].

Another theory suggests that IgE autoantibodies can self inflict direct damage to a tissue, participating in immune response that results in activation of basophils and mast cells. The damaged tissue and release of inflammatory mediators in turn can also result in activation of the urticaria and release of CRP [15]. Kessel *et al* demonstrated a considerable relation between increased total IgE and CSU severity. In the study they explain that this association may be connected to the effects of IgE on mast cell activation and degranulation [16].

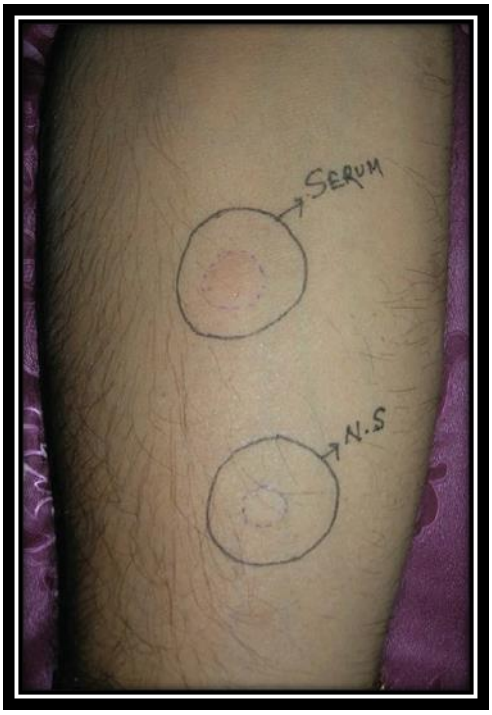

Figure 1

Positive autologous serum skin test. Serum-induced weal is larger than the normal saline (N.S.)–induced wheal.

Chronic autoimmune urticarial and thyroid disease

As remarked previously there is a strong association between autoimmune thyroid disease or thyroid dysfunction and CU, it is the most frequently described autoimmune condition among the patients, where autoimmune hypothyroidism has the largest numbers. The prevalence among adults is 30% [17].
The thyroid disease may worsen urticaria and angioedema through direct mechanisms.
It is thought that in patients with the coexistent diseases there is increased risk for developing angioedema. According to sources it is 16 times more prevalent in patients with thyroid autoimmunity then those without antibodies, 35% will develop episodes of angioedema [18, 19].
In other cases like in a study by Vincenzo Nuzzo *et al* was found that presence of thyroid antibodies did not impair the clinical development of the disease in their group of patients, no observation of different clinical features between patients with and without thyroid autoantibodies was seen [20].

Yet, due to the high prevalence of thyroid disease in patients with CSU, tests for anti-thyroid antibodies and thyroid function should be done in all CSU subjects in order to reveal early on those patients that will need follow-up and treatment.

CSU and other autoimmune disease

Apart from thyroid disease, a variety of other autoimmune disorders have been investigated for connection with CU. Confino- Cohen *et al* found that 12.5% of CU cases had one other autoimmune disease, 2.1% had two diseases, and 0.1% had three diseases. After autoimmune hypothyroidism the most prevalent autoimmune disorder was rheumatoid arthritis (RA) [21]. Another disease with high risk of development was systemic lupus erythematous (SLE). Specific antibody that is used to screen for autoimmunity in disorders like this is antinuclear antibody (ANA).

For assessment of disease severity, in one study was shown that in a positive ANA titers, the percentage of patients with more severe disease course was 50% compared to 30% in those with negative ANA titers [22]. Yet other researchers found no correlation between ANA titer and severity, Levy *et al* in his analysis found that the ANA positivity in CU has rarely even been studied [23].

ANA even seems to have false positives results in 5% - 20% among healthy individuals without any underlying systemic or autoimmune diseases [24].

In conclusion, all these cases show that CSU is a part of a bigger group of diseases, and while many factors may contribute to the pathogenesis, being either autoimmune markers, APR markers or peripheral blood biomarkers, the activity of the disease is still not attributed to a specific test result, the pathogenesis is not yet fully understood and more researches are now being conducted to understand this matter.

RESEARCH METHODOLOGY AND METHODS

Subjects

This study is a retrospective analysis of medical records and laboratory test results of 48 patients with CSU (18 males and 30 females, mean age 39.9 ± 17.0). The patients were recruited from the Department of Immunology and Allegology, Hospital of Lithuanian University of Health Sciences Kaunas Clinics. The study protocol was approved by the Regional Ethics Committee for Biomedical Research, Lithuanian University of Health Sciences. Patients with CSU had a clinical history of continuous or recurrent hives more than 6 weeks. Patients with CSU were divided into 3 groups according to severity of the disease, based on EAACI/GA2 LEN/EDF guidelines of urticaria activity score 7 (UAS7): mild urticaria (UAS7 = 7–15), n=24, moderate urticaria (UAS7 = 16–27), n=13, and severe urticaria (UAS7 = 28–42) , n=11. According the autologous serum skin test (ASST) results, all the patients were divided into 2 groups: CSU patients with negative ASST (n=21), and CSU patients with positive ASST (n=9). None of studied patients had any clinical symptoms of infections.

Laboratory tests

Serum CRP levels were measured using high-sensitivity CRP reagent kits (Dade Behring, USA) with a protein analyzer BNTM100 N. The lower detection limit was 0.15 mg/L. Serum levels of ≤3.10 mg/L were defined as normal [25].

The serum for ASST was prepared by collecting whole blood and centrifuged at 1,250 g for 10 minutes. Intradermal testing with 0.05 mL of autologous serum were performed according to guidelines [26] and read after 30 minutes intradermal injection. A positive result was defined as the appearance of a serum induced wheal 1.5 mm thick or more than the saline-induced response at 30 minutes. ELISA (Enzyme-Linked Immuno Sorbent Assay) method (automatic analyzer Gemini, STRATEC Biomedical systems, Germany) was used to measure the concentration of autoantibodies to TPO (thyroid peroxidase) in serum. Antinuclear antibodies (ANA) detected by indirect immunofluorescence assay method.

Statistical analysis

Statistical analysis was performed by using the Statistical Package for Social Sciences, version 20.0 for Windows (SPSS 20.0). The normality assumption of data was verified with the *Kolmogorov-Smirnov* test. All the data that were normally distributed are presented as mean and standard deviation. The data that did

not follow a normal distribution were expressed as median and interquartile range. Due to a skewed distribution of the variable nonparametric tests, the *Kruskal-Wallis* test was used to evaluate statistical differences between groups. If statistical differences were detected differences between two independent groups were determined by *Mann-Whitney U* test. The categorical data were compared using the chi-square test. Statistical significance was assumed at a P value <0.05.

RESULTS

Characteristics of studied subjects

A total of 48 adults (18 men and 30 women, mean age 39.9 ± 17.0 years) patients with CSU were assessed. Clinical characteristics of the subjects are presented in Table 1. According urticaria activity score 7, patients with chronic spontaneous urticaria were divided into 3 groups: 24 patients with mild chronic spontaneous urticarial (CSU), 13 with moderate CSU, 11 with severe CSU. Angioedema occurrence in patients with CSU was 23%. Factors which may exacerbate the existing urticaria are nonsteroidal anti-inflammatory drugs (NSAIDs) and angiotensin-converting-enzyme inhibitors (ACE inhibitors). The demographic characteristics of the subjects are presented in Table 2. There were no significant age and gender differences comparing the groups. The duration of symptoms was similar in in all groups of patients.

Table 1. Clinical characteristics of the study subjects

Characteristic	Number of patients (%)
Chronic spontaneous urticaria	
Mild	24 (50)
Moderate	13 (27)
Severe	11(23)
Chronic spontaneous urticaria	
With angioedema	11 (23)
Without angioedema	37 (77)
Provoking factors	
Angiotensin-converting-enzyme inhibitors	2 (4)
Nonsteroidal anti-inflammatory drugs	7 (15)

Table 2. Demographic characteristics of the subjects

Characteristic	Patients with mild CSU (n=24)	Patients with moderate CSU (n=13)	Patients with severe CSU(n=11)
Age (yr), median (IQR)	36.0 (31.3)	33.0 (28)	34.5 (21.3)
Sex (male/female), n	13/11	3/10	2/9
Duration of symptoms (yr), median (IQR)	1.5 (4.4)	1.0 (1.5)	1.75 (2.3)

Angioedema is a frequent symptom of CSU. There was no significant differences in presence of angioedema and severity of CSU (P>0.05) Table 3. There also were no significant differences of the rates of autoimmune biomarkers (ANA, anti-TPO or both) in peripheral blood of CSU patient with or without angioedema.

Table 3. Frequency of angioedema in patients with of chronic spontaneous urticaria

Angioedema	Chronic spontaneous urticaria			
	Mild	Moderate	Severe	
Yes (n=11)	7 (29.2%)	2 (15.4%)	2 (18%)	11 (23%)
No (n=37)	17 (70.8%)	11 (84.7%)	9 (82%)	37 (77%)

Markers of inflammation in peripheral blood

There were no significant differences in the peripheral blood neutrophil, lymphocyte and eosinophil counts and immunoglobulin E (IgE) levels in all the groups of studied patients (Table 4).

Table 4. Peripheral blood cells and serum immunoglobulin E levels in patients with CSU

Characteristic	Patients with mild CSU (n=24)	Patients with moderate CSU (n=13)	Patients with severe CSU (n=11)
Total IgE (IU/mL), median (IQR),	3.0 (52.0)	5.4 (200.0)	12.0 (163.6)
Lymphocytes (x109L) median (IQR),	1.3 (0.7)	2.0 (0.5)	1.9 (0.6)
Eosinophils (x109L) median (IQR),	0.2 (0.2)	0.1 (0.2)	0.1 (0.1)
Neutrophils (x109L) median (IQR),	2.9 (1.1)	3.2 (0.8)	3.4 (1.0)

The mean value of serum CRP in all patients was 2,0 (4,6) mg/L , elevated serum CRP levels was found in 10 patients (20.8%). Serum CRP levels was significantly higher in patients with severe CSU than those with mild and moderate CSU (P<0.05) Figure 1. There was no significant difference in serum CRP levels comparing patients with mild and moderate CSU.

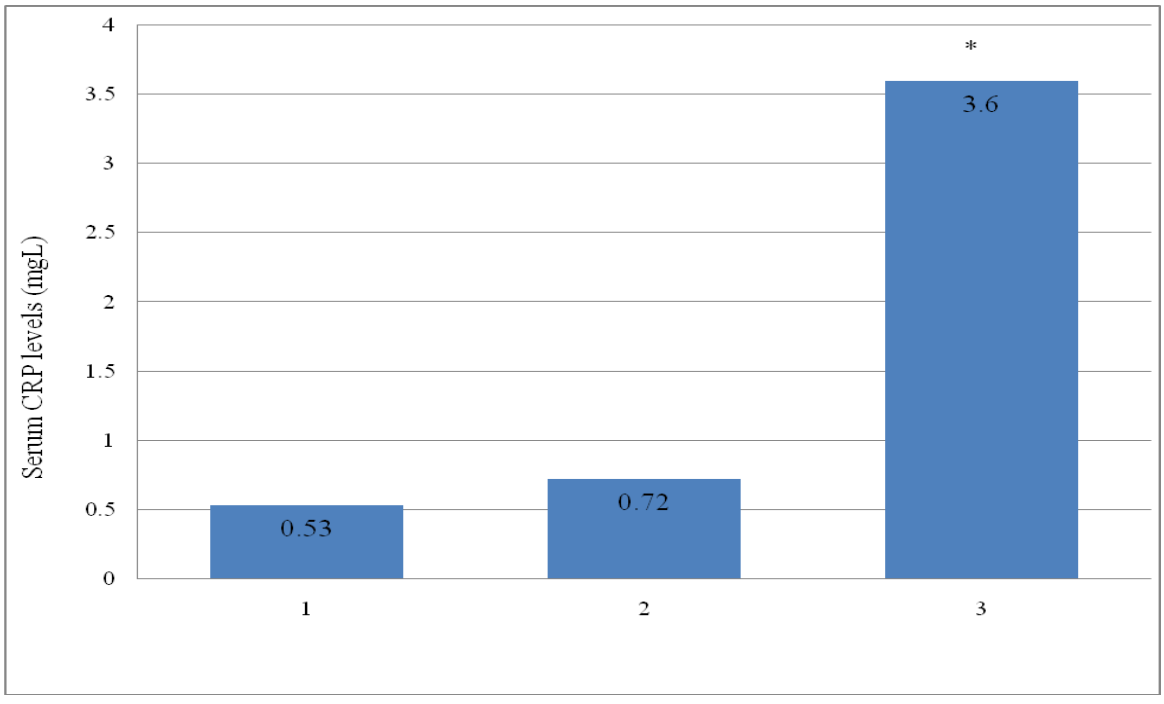

Figure 1. Serum C-reactive protein levels in subject with chronic spontaneous urticaria 1-mild (n=24); 2-moderate (n= 13); 3-severe (n=11); Data are expressed as median;
*P<0.05, versus patients with mild and moderate chronic spontaneous urticaria.

There were no significant differences between autoimmune biomarker (ANA and anti-TPO) in peripheral blood and course of CSU (Table 5).

Table 5. Autoimmunity markers in peripheral blood of patients with chronic spontaneous urticaria

	Chronic spontaneous urticaria		
	Mild*	Moderate	Severe
ANA Positive/Negative	4 /20	4/9	3/8
Anti –TPO Positive/Negative	7/17	3/10	3/8

*P<0.05.

Assessment of autoimmunity in patients with chronic spontaneous urticaria

Autologous serum skin test (ASST) was done on 30 patients: Patients with positive ASST showed higher UAS7 comparing with ASST negative patients 38.1(7.2) vs 20.0(10.4), P <0.05).

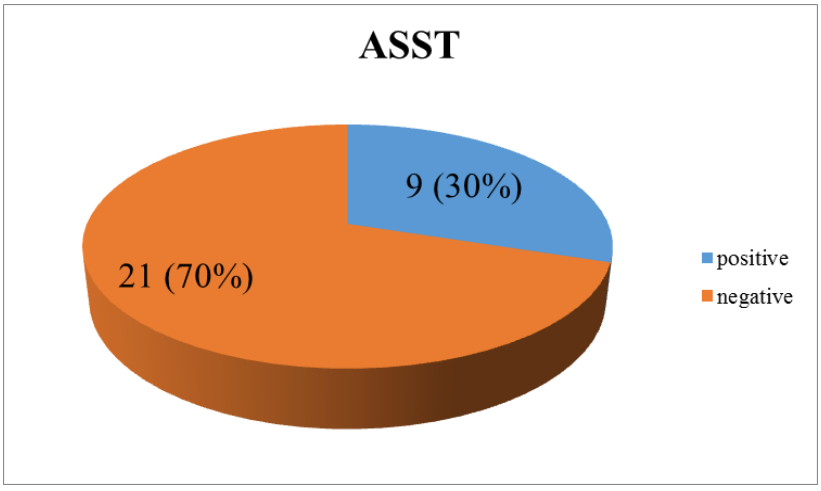

Figure 2. Distribution of autologous serum skin test positive and negative subjects

The results shows, that from 9 patients with positive ASST, 3 had overlap with both ANA and anti-TPO positive (P<0.05), 3 patients had either ANA or anti-TPO antibodies, and 3 patients didn't have any antibodies (Table 6). There were no significant differences between autoimmune biomarker (ANA and anti-TPO) in peripheral blood and severity of disease in patients with positive or negative ASST (P>0.05). Serum CRP levels, peripheral blood cell count and IgE levels did not show any significant differences in CSU patients with positive and negative ASST.

Table 6. Autoimmunity markers in peripheral blood of patients with positive and negative autologous serum test

ASST **(%)**	ANA		ATPO *	
	positive	negative	positive	negative
Positive (n=9)	4 (44.4%)	5(55.6%)	5 (55.6%)	4 (44.4%)
Negative (n=21)	5 (23.8%)	16 (76.2%)	3 (14.3%)	18 (85.7%)

*P<0.05.

There was no significant differences in presence of angioedema in CSU patients with positive or negative ASST (P>0.05) Table 7.

Table 7. Frequency of angioedema in chronic spontaneous urticaria patients with positive and negative autologous serum test

ASST **(%)**	Angioedema	
	yes	No
Positive (n=9)	2 (22.2%)	7 (77.8%)
Negative (n=21)	3 (14.3%)	18 (85.7%)

DISCUSSION OF THE RESULTS

The aim of the research was to investigate CRP levels in CSU patients and discover possible connection between CRP and disease activity in order to get some idea on pathogenesis of urticaria or understand disease activity. As previously stated, CRP in relation to the activity of the disorder implies that the marker can be used as a diagnostic tool. Investigating it can help establish a basis for pathogenesis [6]. First main result shows that higher serum CRP levels are found in patients with more severe course of the disease compared to mild or moderate course ($p<0.05$). A study by Takahagi *et al* also found that patients with severe CSU had elevated CRP concentration more than patients with moderate or mild disease activity [9].

In a reviewed study by Rabelo-Filardi *et al* on spontaneous chronic urticaria, he concluded that the clinical activity of CSU can estimate disease duration, and elevation of different parameters in the serum including CRP functions as markers for activity of the disease [27].

Another study that supports our findings showed that the elevated CRP being an acute phase reactant, may enhance urticarial inflammation and thus disease activity in CU [28].

In many patients with CSU, no definitive etiology can be found. In such cases, autoimmunity is considered. The presence of mast cell stimulation and histamine release is demonstrated with the ASST.

Other tests in our study were looking for connection of autoimmunity and disease severity. No significant difference was found to activity of urticaria with ANA or anti-TPO marker.

The next test analyzed ASST positivity in 30 patient to find different relation to autoimmunity. It was shown that 9 patients (30%) had positive ASST belonging to CAU class. From them 3 patients did not have positive markers for ANA or anti-TPO. This could mean that other autoimmune systemic disorders are responsible for the CSU. On the other hand from 21 (70%) negative ASST patients 6 were found to have positive autoimmune markers. This could mean that not everyone with autoimmunity is ASST positive, and they remain in the category of CIU.

Another explanation to the association of ASST and autoimmune disease can be that the disease activity of CU is unpredictable, it has relapses and remissions. There are no definitive clinical features or blood marker or guideline criteria that can say that patient is in permanent remission. Therefore some ASST positive patients with associated thyroid autoimmunity can at some point be ASST negative when the antibody titers goes down while the autoimmunity itself persists [29].

In this study there was no significant differences between autoimmune biomarker (ANA and anti-TPO) in peripheral blood and severity of disease in patients with positive or negative ASST (P>0.05).
In the literature one research looked for association between thyroid autoimmunity and CAU, they found that ASST positive and ASST negative patients did not have specific diagnostic clinical feature and thyroid autoimmunity was found to be equally associated with the both positive and negative ASST groups [29]. In other study in relation between disease activity and ASST positivity was found that statistical analysis of patients did not show a significant difference between patients with positive and negative ASST regarding the disease duration and severity [30]. Yet in our study it was found in the results that patients with positive ASST showed higher UAS7 comparing with ASST negative patients (P<0.05). In regards to serum CRP levels, there was no significant differences in CSU patients with positive and negative ASST and elevation of CRP.

Patients with autoimmune urticaria frequently have associated angioedema and the urticaria tends to run a more chronic course [31]. It is thought that in patients with the coexistent diseases there is increased risk for developing angioedema. In a study by Missaka *et al* angioedema was detected in 70 patients (60.9%) with CSU. Patients with CSU and autoimmune thyroid disease present greater risk of angioedema, which strengthened the idea that a relationship exists between this condition and thyroid autoimmunity [18]. In this research 11 patients (23%) have presence of angioedema but there was no significant differences in presence of angioedema and activity of CSU (P>0.05). There also were no significant differences of the rates of autoimmune biomarkers (ANA, anti-TPO or both) in peripheral blood of CSU patient with or without angioedema.

In other domains, analysis of peripheral blood neutrophil, lymphocyte, eosinophil counts and immunoglobulin E (IgE) levels were assessed in relation to activity of urticaria and no significant difference between the groups was found.
One study evaluated the usefulness of some laboratory markers in assessing disease severity, IgE was not found to be reliable predictor for disease activity [32]. Yet other studies were conducted on the same topic and they came to the opposite conclusion. Kessel *et al* demonstrated a considerable relation between increased total IgE and CU severity. In the study they explain that this association may be connected to the effects of IgE on mast cell activation and degranulation [16].
Another test looked for IgE levels in comparison to eosinophils levels. Low-affinity IgE receptor are found on eosinophils membranes. They have been identified in approximately 65% of CSU

patients. These antibodies can trigger eosinophils, inducing the release of major basic protein, which in turn causes mast cell degranulation [33]. In our results, no patient had both elevated markers. In other resources it is also said that IgE levels and eosinophil percentages are not good indicators for a prolonged course of urticaria [34].

Lastly, regarding provoking factors of the disease. Drugs are known to induce urticaria. Avoidance of aspirin and other NSAIDs is recommended because these drugs aggravate chronic urticaria in about 30% of patients [35, 36]. ACE inhibitors in turn are dangerous because of high prevalence of angioedema [37]. In this research overall 9 patients (19%) were using NSAID's or ACE inhibitors during flaring of the disease.

CONCLUSIONS

1. Chronic inflammation is common in patients with chronic spontaneous urticaria, the prevalence of patients with increased serum CRP levels is 20.8%.

2. Serum C- reactive protein levels were significantly higher in patients with severe chronic spontaneous urticaria, but no significance was found between activity and presence of angioedema.

3. There were no differences in C- reactive protein levels in chronic spontaneous urticaria patients with positive and negative autologous serum skin test.

4. No difference was found between immunity markers (anti-TPO and ANA) to disease activity.

RECOMMENDATIONS

1. We recommend to evaluate CRP levels in patients with CSU to associate it with disease activity, and for follow up of disease activity.

2. Because no association of angioedema was found with disease activity, we recommend to not address the presence of angioedema as a sign for disease severity.

3. We recommend to perform a wider research on the topic of CRP association with CSU, and to find the upper and lower limits of CRP levels in each group activity (mild, moderate, and severe).

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