Vilnius University

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DIAGNOSTIC SIGNIFICANCE OF AUTOREACTIVITY MARKERS IN CHRONIC URTICARIA

Summary of Doctoral Dissertation
Biomedical Sciences, Medicine (07B)

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Anželika Chomičienė

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List of abbreviations

ANA – antinuclear antobodies
ANCA – anti-neutrophil cytoplasmic antibodies
AH – antihistamines
ASST – autologous serum skin test
BD – Becton Dickinson
BSB – basophil stimulation buffer
CAU – chronic autoimmune urticaria
CD – cluster of differentiation
CIU – chronic idiopathic urticaria
CU – chronic urticaria
EAACI – European Academy of Allergology and Clinical Immunology
ELISA – enzyme linked immunosorbent assay
FcεRI – high-affinity immunoglobulin E receptor
fMLP – N-Formil-Met-Leu-Phe chemotactic peptide
FITC – fluorescein isothiocyanate
HLA – human leucocyte antigens
HRA – basophil histamine release assay
IgE – immunoglobulin E
NSAID – non-steroidal anti-inflammatory drug
TPO – thyroid peroxidase antibodies
PBS – phosphate buffer saline
PE – phycoerithrin
PerCP – peridinin-chlorophyll-protein
SD – standart deviation

Table of Contents

1 Introduction

Chronic urticaria (CU) is a common skin disorder characterized by recurrent appearance of wheals and/or angioedema for more than 6 weeks. CU affecting up to 1% of the population and associated with severely impaired quality of life. It remains a major problem in terms of aetiology, investigation and management. Allergy is rare cause of CU. The etiological reason cannot be found in many cases and the disease is defined as chronic idiopathic urticaria (CIU).

Recently it has become clear that 30 to 50% of patients with CIU have functional autoantibodies directed against the α-chain of the high-affinity immunoglobulin E (IgE) receptor (FcεRI) or less common against IgE. The term chronic „autoimmune urticaria" (CAU) increasingly is used to reflect advances in knowledge about functional autoantibodies that activate mast cells and basophils through cross-linking the FcεRI to secrete histamine (Grattan CE, 2004). Nonfunctional autoantibodies against FcεRI have been detected by immunoassay in healthy subjects but seem to be nonpathogenic.

The finding that HLA subtypes DRB1*04(DR4) and DQB1*(DQ8) are strongly associated with the subset of patients with autoantibodies (O'Donnell BF et al., 1999) is one of the evidences supporting an autoimmune basis of CIU. The association of CU and autoimmune disease has been recognized for some time, especially with autoimmune thyroid disease. The incidence of thyroid antibodies in chronic urticaria varies between 12 and 33%.

Basopenia in patients with CIU has long been recognized and explained by destruction or sequestration of basophils by autoantibodies.

Abnormalities of B and T cells have been described in CU that tend support to the concept of autoimmune disease.

Approximately 30% of patients with CIU experience flares of hives after taking non-steroidal anti-inflammatory drugs (NSAID's). There is an evidence that patients with cross reactive type of NSAID's hypersensitivity had autoimmune nature of CU.

The patients with CAU generally had more severe and difficult-to-control symptoms than those with CIU. An early diagnosis of CAU should optimize the care of these patients and improve the outcome of their disease. The identification of patients with CAU has encourage the use of immunotherapy in patients with severe disease unresponsive to antihistamine therapy. There is an evidence of clinical improvement in parallel with reduced antibody levels after some kinds of immunotherapy.

The only in vivo method, the autologous serum skin test (ASST) is screening test for functional antibodies in CU. A positive test is suggestive, but not diagnostic of autoimmune basis for the patients CU. ASST does not prove the presence of antibodies, but rather shows histamine-releasing properties of the tested serum. The sensitivity and specifity of this test is reported to be about 70 and 80% respectively (Sabroe RA, et al., 1999).

Techniques to detect the autoantibody to FcεRI and IgE *in vitro* include binding assays Western blot and ELISA, but they fail to identify antibodies with histamine releasing properties. The basophil histamine release assay (HRA), currently the "gold standart" for detecting functional antibodies in the serum of patients with CU, is difficult to standardize because of differences in donor basophils, which are used for the test and

methodological differences. The HRA sensitivity and specifity showed by some authors is not much better as ASST. Furthermore, HRA is time consuming and cumbersome to perform.

There is also still no simple, reproducible clinical test for functional antibodies. Improved screening tests are being sought; for example, ability of CU patient's serum to evoke expression of CD63 and CD203c on donor human basophils by using flow cytometry is showing some promise. There are 5 published studies about using CD63 expression as diagnostic tool in CAU and only 1 small study (Yasnowsky KM, et al., 2006) which was done by using CD203c expression on basophils from atopic donor. CD203c appears to be selectively expressed on the basophils/mastocyte lineage and may be a better basophil activation marker. There are no studies showing comparison of both CD63 and CD203c expression tests in CU. In Lithuania we have no studies in terms of CAU.

2 The aim of the study

To evaluate the diagnostic significance of autoreactivity markers and their correlation in patients with chronic urticaria.

3 Tasks of the present study

1. To assess the affect of sera from patients with CU and healthy controls on basophil CD63 and CD203c expression.
2. To estimate the cut-off value of the CD63 and CD203c expression tests for CAU diagnosis.
3. To estimate the sensitivity and specifity of the CD63 and CD203c expression tests and their diagnostic significance for CAU.
4. To estimate the correlation of the CD63 and CD203c expression tests with ASST and internecine correlation.
5. To estimate the correlation of markers of CAU (ASST, CD63, CD203c expression) with other autoreactivity indicators (thyroid peroxidase antibodies (TPO), antinuclear antobodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA)).
6. To estimate the correlation of CAU markers with CU activity, length of the disease, angioedema, sensitivity to NSAID's and treatment efficacy.

4 Significance and novelty of research work

1. CD63 and CD203c expression tests were performed and compared as diagnostic markers of CAU in large series of patients.
2. CD63 and CD203c expression tests sensitivity and specifity were estimated with using of different cut-off values for CAU diagnosis.
3. The correlation between CD63 and CD203c expression tests and ASST was estimated and diagnostic value of the tests for CAU was evaluated.
4. The correlation of CD63 and CD203c expression tests with other autoreactivity indicators CU was evaluated.

5. The correlation of CD63 and CD203c expression tests with some CU features was evaluated.

5 Materials and methods

5.1 Patients and controls

128 patients with diagnosis of chronic urticaria were investigated (26 males and 102 females). Mean age of patients: 43±13 years (age range: 20–78 years). The patients with physical urticaria only were exluded from the study.
30 healthy controls: 15 males ir 15 females, mean age 30±7 years (age range 22–51 years) took part in the study. All participants provided inform consent.

5.2 Study tests

The ASST was performed in all patients and 10 controls. CD63 and CD203c expression tests were performed in all patients and controls.
TPO were detected in all CU patients, TSH was established in all patients with elevated and 48 patients with normal TPO.
ANA, ANCA were detected in 83 CU patients.
116 patients answered the European Academy of Allergology and Clinical Immunology/ European Union–funded network of excelence/ European Dermatology Forum (EAACI/GA²LEN/EDF) questionnary.
All patients were interviewed about sensitivity to NSAID‘s and conventional treatment with second-generation antihistamines efficiacy.

5.2.1 Autologous serum skin test

Antihistamines were avoided 5 days before the skin testing.
The test was performed by injecting 0.05 ml of the patient‘s own serum intradermally into the volar aspect of the forearm. Sterile saline was used as negative control. A skin prick test with histamine 10 mg/ml was carrried out as positive control to exclude any residual effect of anti-histamine drugs. Wheal and flare reactions were measured at 30 and 60 minutes. A mean wheal diameter of at least 1.5 mm greater than negative control with saline was considered to be positive ASST.

5.2.2 Basophil activation tests

Basophil donor

The basophil donor was atopic and had serum IgE level of 1350 U/ml. The basophil donor provided inform consent and was bled less than 1 time per week.

5.2.2.1 Measurment of CD203c surface expression

The test was performed with heparinized blood within 3 to 4 hours after drawing the donor‘s blood. Aliquots of the donor‘s heparinized whole blood (200 µl) were incubated for 20 minutes at 37ºC with 40 µl sera from patients with CU or normal controls and 40 µl basophil stimulation buffer (BSB) (Becton Dickinson (BD), USA). For controls, 40 µl phosphate buffer saline (PBS) was added and used as negative control, and 40 µl -µM N-Formil-Met-Leu-Phe chemotactic peptide (fMLP) (Sigma, USA) and 40 µl of a 1:50 dilution of anti-FcεRI receptor antibody (Upstade, USA) in Ca^{++} Mg^{++} free PBS were used as positive control. The reactions were stopped by placing the tubes on ice for 5 min. Cells were stained with phycoerithrin (PE)-conjugated antihuman CD45 (BD, USA), peridinin-chlorophyll-protein (PerCP)-antihuman CD203c (Immunotech, USA), fluorescein isothiocyanate (FITC)-conjugated antihuman IgE (Invitrogen, USA) at room temperature in the dark for 30 minutes. Red cells were lised with 2 ml FACS Lysing Solution (BD, USA). The cells were washed once with 2 ml PBS and fixed in 0.5% paraformaldehyde. The cells were analyzed on a FACSCalibur flow cytometer (BD). Data on at least 1000 basophils were acquired, and the percentage of CD203c-expressing basophils was calculated.

5.2.2.2 Measurment of CD63 surface expression

Test was performed with standart BD FastImmune set.
The test was performed on heparinized blood within 3 to 4 hours after drawing the donor‘s blood. Aliquots of the donor‘s heparinized whole blood (100 µl) were incubated for 20 minutes at 37ºC with 100 µl sera from patients with CU or normal controls and 20 µl BSB. For controls, 100 µl PBS was added and used as negative control, and 100 µl 1-µM fMLP was used as positive control. The reactions were stopped by placing the tubes on ice for 5 min. Cells were stained with 20 µl CD63FITS/ CD123PE/ Anti-HLA DR PerCP antibodies coctail at room temperature in the dark for 30 minutes. Red cells were lised with 2 ml FACS Lysing Solution. The cells were washed once with 2 ml PBS and fixed in 0.5% paraformaldehyde. The cells were analyzed on a FACSCalibur flow cytometer (BD). Data on at least 1000 basophils were acquired, and the percentage of CD63-expressing basophils was calculated.

5.3 Statistical analysis of results

Statistical significance was determined by using the Wilcoxon matched pairs test, the Man-Whitney test, or χ^2 test, where appropriate. A p value of less than 0.05 was considered as significant. Statistical analysis of results was performed with SSPS 15 program.

6 Results

6.1 CD63 and CD203c expression tests results

We found, that on average sera from CU patients significantly upregulated CD63 and CD203c expression on atopic donor basophils. CD63 and CD203c expression assay results in patients and controls are shown in Table 1.

Table 1. Basophils activation (% of cells expressing markers) with patients and controls sera

	Patients (N=128)		*Controls* (N=30)	
	CD203c	CD63	CD203c	CD63
Mean	**11.79**	**10.77**	**1.84**	**3.10**
Standart deviation (SD)	12.4	10.9	0.5	1.5
Median	8.2	8.1	1.7	3.0
Min	0.6	0.1	1.0	0.7
Max	65.8	60.5	2.7	6.9

For comparison the mean values of positive and negative controles are presented in Table 2.

Table 2. Basophil activation (% of cells expressing markers) after positive (fMLP) and negative (buffer) control

	CD63 (N=16)		*CD203c* (N=16)	
	Positive control	Negative control	Positive control	Negative control
Mean	**40.0**	**4.4**	**35.6**	**4.6**
SD	4.3	0.6	5.7	0.8
Median	38.1	4.2	38.4	4.0
Min	11.8	1.4	9.3	1.1
Max	70.8	8.5	84.7	9.2

Kolmogorov-Smirnov test showed, that CD63 ir CD203c data are not normally distributed (p=0.00). Therefore, we used Mann-Whitney test to compare the data (Table 3).

Table 3. Comparison of CD63 ir CD203c expression in patients and controls (Mann-Whitney test)

	CD203c	*CD63*
Mann-Whitney U	777.500	883.500
Wilcoxon W	1242.50	1348.50
Z statistics	–5.066	–4.595
p	0.000	0.000

Mann-Whitney test showed there are significant differences in patients and controls groups in both (CD63 and CD203c) expression tests (significance values for both tests were 0.000). All data are presented in Figures 1 and 2.

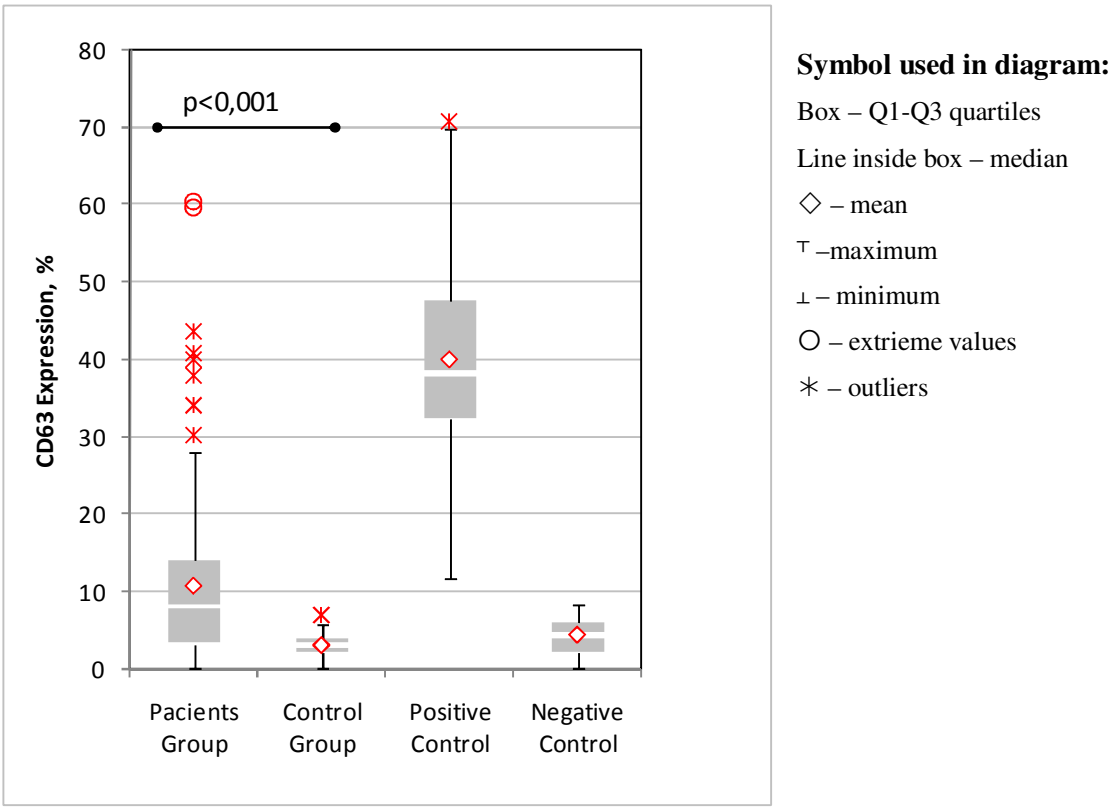
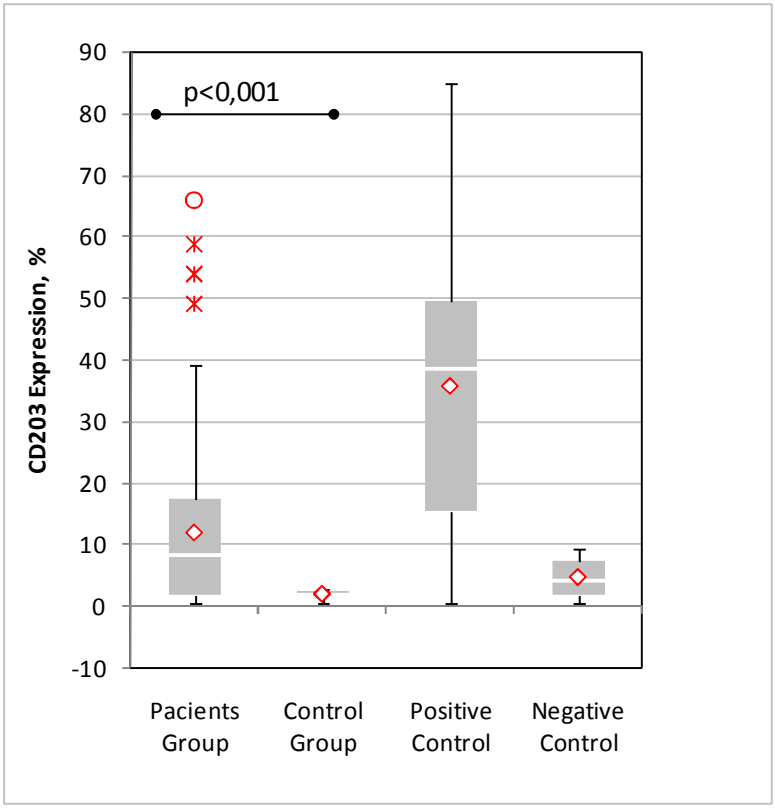


Figure 1. CD63 expression (%) in patients and control group, positive and negative control



Symbol used in diagram:

Box – Q1-Q3 quartiles

Line inside box – median

◇ – mean

⊤ –maximum

⊥ – minimum

○ – extrieme values

✳ – outliers

Figure 2. CD203c expression (%) in patients and control group, positive and negative control

6.2 The investigation of cut-off values of the CD63 and CD203c expression tests

Seven most often used for basophil activation tests cut-off levels were evaluated: mean value of control group + 2 SD; mean value of control group + 3 SD; 5%; 10%; 15%; twice PBS stimulation; 95th percentile above control sera induced CD63 or CD203c expression. Cut-off values are presented in Table 4.

Table 4. Cut-off values

	Cut-off value 1	Cut-off value 2	Cut-off value 3	Cut-off value 4	Cut-off value 5	Cut-off value 6	Cut-off value 7
	Mean value of control group + 2 SD	Mean value of control group + 3 SD	15%	10%	5%	2x negative control	95th percentile
CD203c	2.88	3.40	15	10	5	NA	2.700
CD63	6.04	7.52	15	10	5	NA	6.262

NA – not applicable

Sensitivity and specifity of the CD63 and CD203c tests were calculated according to the formula of Galen and Gambino, using the ASST as „gold standard“. The data of sensitivity, specifity and tests positivity depending on the different threshholds are shown in Tables 5 and 6.

Table 5. Sensitivity, specifity and positivity of CD63 expression assay

	Cut-off value 1	Cut-off value 2	Cut-off value 3	Cut-off value 4	Cut-off value 5	Cut-off value 6	Cut-off value 7
	Mean value of control group + 2 SD	Mean value of control group + 3 SD	15%	**10%**	5%	2x negative control	95 percentile
CD63 (positive, %)	61.7	56.3	21.9	**36.7**	65.6	49.2	60.9
sensitivity	74.4	74.4	37.2	**55.8**	79.1	67.4	74.4
specifity	44.7	52.9	85.9	**72.9**	41.2	60.0	45.9

Table 6. Sensitivity, specifity and positivity of CD203c expression assay

	Cut-off value 1	Cut-off value 2	Cut-off value 3	Cut-off value 4	Cut-off value 5	Cut-off value 6	Cut-off value 7
	Mean value of control group + 2 SD	Mean value of control group + 3 SD	15%	**10%**	5%	2x negative control	95 percentile
CD203c (positive, %)	64.1	63.3	33.6	**45.3**	59.4	39.1	64.8
sensitivity	67.4	67.4	41.9	**55.8**	65.1	44.2	67.4
specifity	37.6	38.8	70.6	**60.0**	43.5	63.5	36.4

The cut-off value giving the best sensitivity and specifity (55.8% and 72.9% for CD63 expression, 55.8% and 60.0% for CD203c expression respectively) was found to be 10%. Additionally, to obtain specific cut-off point for significant percentage of activated basophils, sensitivity and specifity were evaluated by using receiver operator characteristic (ROC) curve analysis. The data are shown in Table 7 and Figure 3.

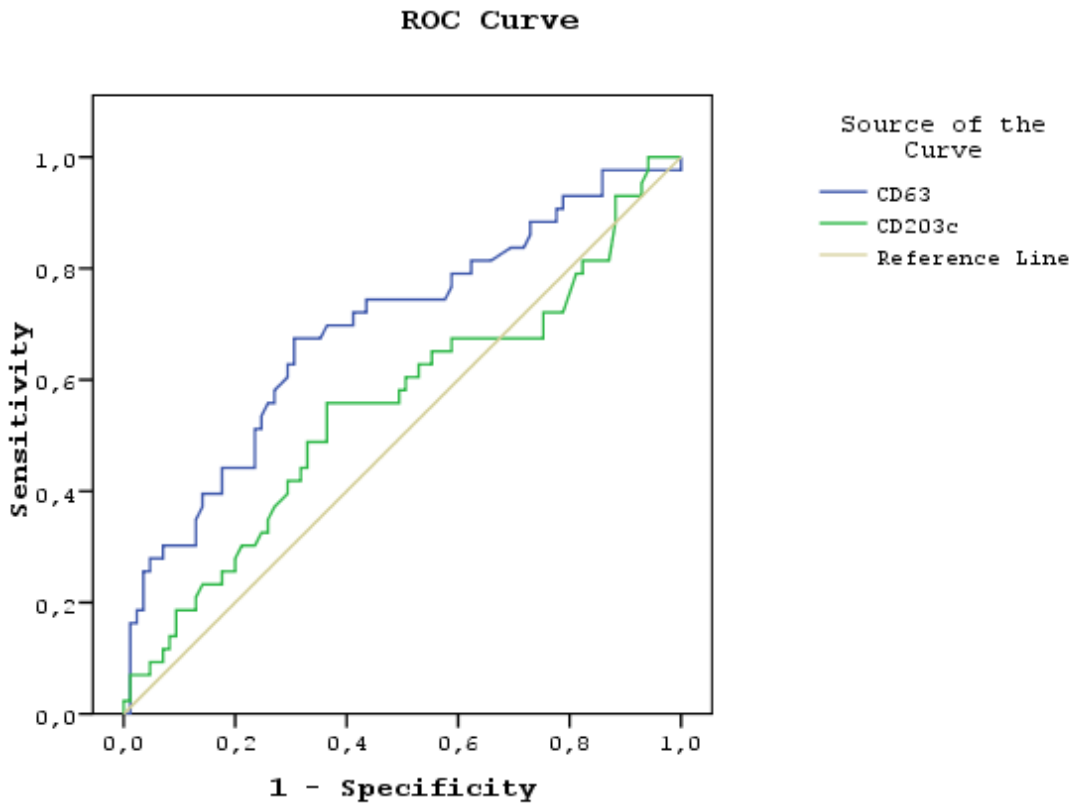


Figure 3. CD63 ir CD203c ROC curves

Table 7 . Area under the ROC curve

Test Result Variable(s)	Area	Std. Error(a)	Asymptotic Sig.(b)	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
CD63	0.691	0.051	0.000	0.591	0.791
CD203c	0.552	0.056	0.339	0.442	0.662

As the CD203c area under ROC curve was about 0.5 and p>0.05, we concluded that the CD203c had not sufficient prognostic features, but we used it for experimental purpose in further investigations.

We applied the calculation of Youden index, total accuracy and total misclassification errors values for CD63 expression test with intention of maximizing accuracy and minimizing misclassification errors of the test and to establish the correct cut-off value. Results of the tests are presented in Table 8.

Table 8. Youden index ir total misclassification error index rezults (%)

	Cut-off value 1	Cut-off value 2	Cut-off value 3	Cut-off value 4	Cut-off value 5	Cut-off value 6	Cut-off value 7
	Mean value of control group + 2*SD	Mean value of control group + 3*SD	15%	**10%**	5%	2*Negative control	95 percentile
CD63 (positive, %)	61.7	56.3	21.9	**36.7**	65.6	49.2	60.9
sensitivity	74.42	74.42	37.21	**55.81**	79.07	67.44	74.42
specifity	44.71	52.94	85.88	**72.94**	41.18	60.00	45.88
Youden index	19.13	27.36	23.09	**28.75**	20.25	27.44	20.3
Total accuracy	54.68	60.2	69.5	**67.2**	53.9	62.5	55.5
TME0	80.9	72.6	76.9	**71.2**	79.8	72.6	79.7
TME1	45.3	39.8	30.5	**32.8**	46.1	37.5	44.5

The estimated best cut-off value of CD63 test was **10%**. We used this threshold for both CD63 and CD203c in further investigations.
Considering the estimated cut-off, we found that sera from 36.7% patients induced upregulation of CD63 and sera from 45.3% of patients upregulated CD203c molecule.
Only Frezzolini A. et al. (2006) used ROC curve analysis for CD63 expression test in aim to establish cut-off able to discriminate ASST positive and negative CU patients. The cut-off giving the best sensitivity and specifity (95% and 91% respectively) was found to be 15%. We can explain this discrepancy with our study by different flow cytometry technics used in the studies. Frezzolini A. et al. used a tricolour flow cytometric method, which allows more accurately quantify activated CD63 cells.

6.3 ASST results and correlation with basophil activation tests

ASST was positive in 43 (33.6%), negative – 85 (66.4%) patients (Figure 4). This data are in accordance with most investigators, the estimated ASST positivity is 30–60%. ASST performed on the healthy control group (n=10) was negative in all subjects.

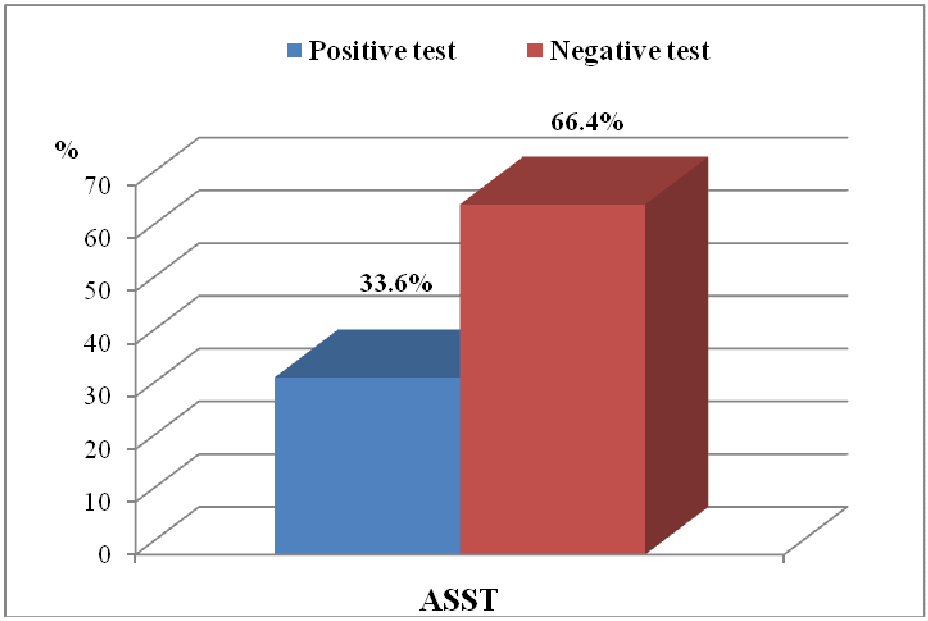


Figure 4. ASST results

The relationship between basophil activation test and ASST is shown in Table 9.

Table 9. The relationship between basophil activation tests and ASST

	ASST					
	Positive		Negative		Total	
	N	%	N	%	N	%
CD203c						
Positive	24	55.8	34	40.0	58	45.3
Negative	19	44.2	51	60.0	70	54.7
Total	43	100.0	85	100.0	128	100.0
CD63						
Positive	24	55.8	23	27.1	47	36.7
Negative	19	44.2	62	72.9	81	63.3
Total	43	100.0	85	100.0	128	100.0

24 (55.8%) patients with positive and 23 (27.1%) with negative ASST had elevated CD63 expression. 24 (55.8%) patients with positive and 34 (40%) with negative ASST had elevated CD203c expression. Possible explanations are that basophils activation tests are more sensitive or that in patients with negative ASST results (with CAU) the dermal mast cells were desensitized by autoantibodies at the time of the ASST. Another explanation is that there are other undefined serum factor present in CU sera that can upregulate CD63 and CD203c. Whereas negative CD63 and CD203c expression was found in 19 (44.2%) ASST positive patients. It can be explained by the existence of a mast cell-specific histamine releasing factor, causing positive ASST. Also, false-positive ASST results could be due bradykinin or C5a generated in serum during clotting.

We found significant correlation between CD63 expression test and ASST ($r= 0.282$, $p = 0.001$), but there was no correlation between CD203c and ASST ($r = 0.150$, $p = 0.091$). Mann-Whitney test showed that CD63 expression was statistically different

in ASST positive and ASST negative groups, but there were no differences in CD203c and ASST groups. The same results were obtained when Pearson's chi-square test was applied (CD63 and ASST: p=0.001, $\chi 2$=10.161, df=1; CD203c: p=0.09, $\chi 2$= 2.882, df=1). Different ASST and CD63 and ASST and CD203c correlations can be explained by differences in methodology of the tests. Most authors, who used CD63 or CD203c expression in CU patients, found significant correlation between ASST and basophil activation tests positivity.

6.4 Correlation between CD63 and CD203c expression tests

The relationsheep between CD63 and CD203c tests are shown in table 10.

Table 10. CD63 ir CD203c expression results

	CD203c				Total	
	Positive		Negative			
CD63	N	%	N	%	N	%
Positive	32	55.2	15	21.4	47	36.7
Negative	26	44.8	55	78.6	81	63.3
Total	58	100.0	70	100.0	128	100.0

Significant correlation between both CD63 and CD203c expression tests was found (p=0.000, $\chi 2$=15.543, df=1). In our knowledge we compared CD63 and CD203c tests in CAU diagnosis for the first time.

6.5 The correlation of CD63, CD203c expression tests and ASST with other autoreactivity markers

6.5.1 Thyroid peroxidase antibodies

TPO were positive in 32 (25%) of patients (Figure 5). The incidence of thyroid antibodies detected by other investigators in CU patients varies between 12 and 33%.

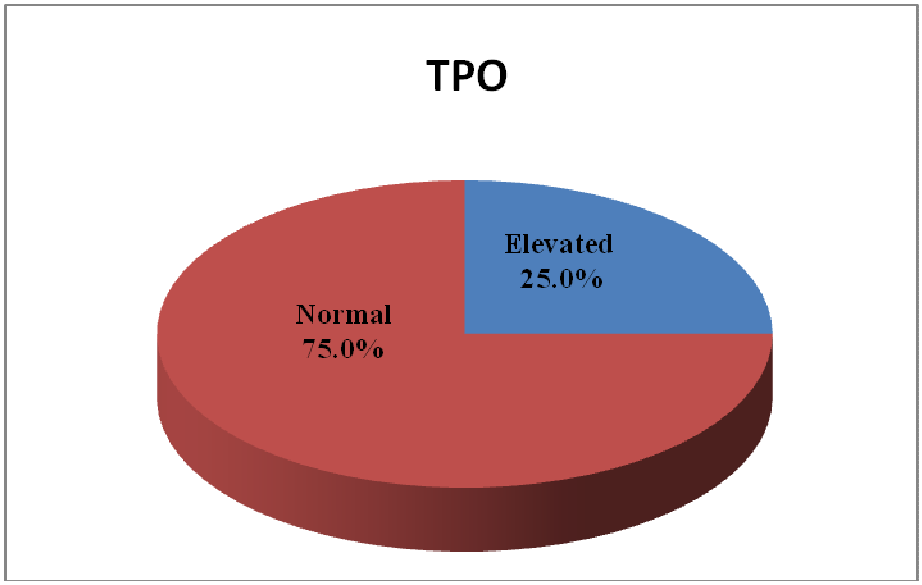


Figure 5. TPO results

TPO and TSH results are shown in Table 11.

Table 11. TPO and TSH results

	TPO				Total	
	Normal		Elevated			
TSH:	N	%	N	%	N	%
Normal	44	91.7	23	71.9	67	83.8
Elevated	0	0.0	4	12.5	4	5.0
Reduced	4	8.3	5	15.6	9	11.3
Total	48	100.0	32	100.0	80	100.0

Elevated TSH, indicating thyroid hypofunction was found only in 4 (12.5%) patients with increased TPO. However, reduced TSH (thyroid hyperfunction) was found in both (elevated – 5 (15.6%) and normal – 4(8.3%) TPO) groups (Figure 6).

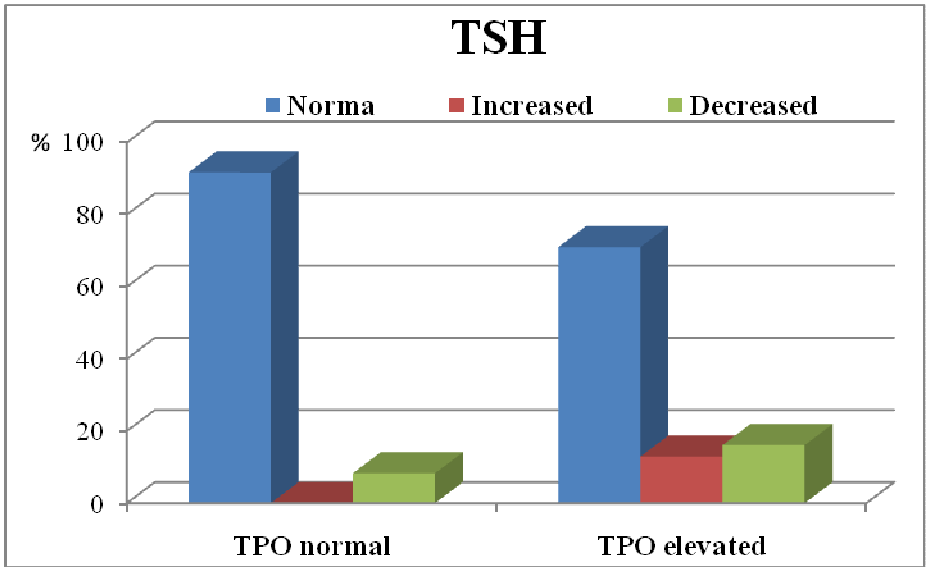


Figure 6. TPO and TSH

The data of relationship between TPO and CAU markers (ASST, CD63, CD203c) are shown in Tables 12–14.

Table 12. TPO and ASST results

	TPO				Total	
	Normal		Elevated			
ASST:	N	%	N	%	N	%
Negative	65	67.7	20	62.5	85	66.4
Positive	31	32.3	12	37.5	43	33.6
Total	96	100.0	32	100.0	128	100.0

Table 13. TPO ir CD203c expression results

	TPO				Total	
	Normal		Elevated			
CD203c	N	%	N	%	N	%
Negative	49	51.0	21	65.6	70	54.7
Positive	47	49.0	11	34.4	58	45.3
Total	96	100.0	32	100.0	128	100.0

Table 14 . TPO ir CD63 expression results

	TPO				Total	
	Normal		Elevated			
CD63	N	%	N	%	N	%
Negative	64	66.7	17	53.1	81	63.3
Positive	32	33.3	15	46.9	47	36.7
Total	96	100.0	32	100.0	128	100.0

Unlike the most investigators, we didn‘t find significant correlation between elevated TPO and positive ASST (p=0.589, χ^2=0.292, df=1). No statistically significant correlation was found between TPO and CD63 expression (p=0.169, χ^2=1.894, df=1), TPO and CD203c expression (p=0.151, χ^2=2.060, df=1). In our opinion CAU and autoimmune thyroiditis are two separate autoimmune events. We think, all patients should be screened for thyroid antibodies and thyroid function for early identification of patients requiring either treatment of underlying thyroid dysfunction or follow-up.

6.5.2 Antinuclear and anti-neutrophil cytoplasmic antibodies

ANA were positive in 7 (8.4%) of patients: one of them had systemic *lupus erythematosus*, in one connective tissue disease was suspected, two had elevated TPO and three of patients had chronic infection focuses. 2 (2.4%) patients had positive ANCA and no underlying diseases. 1 patient without any other than CU pathology was positive on both ANA and ANCA tests. Results are shown on Figure 5.

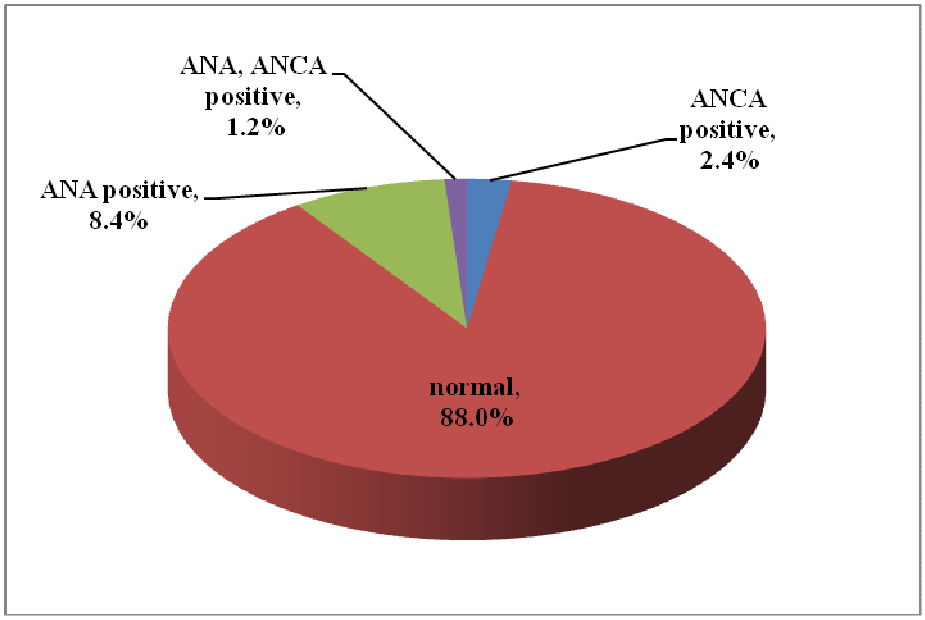


Figure 5. ANA, ANCA results

The relationship between ANA, ANCA and CAU markers (ASST, CD63, CD203c) are shown in Tables 15–17.

Table 15. ANA, ANCA and ASST results

	ASST				Total	
	Positive		Negative			
ANA, ANCA:	N	%	N	%	N	%
ANA positive	6	12.0	1	3.0	7	8.4
ANA and ANCA positive	0	0.0	1	3.0	1	1.2
ANCA positive	0	0.0	2	6.1	2	2.4
Normal	44	88.0	29	87.9	73	88.0
Total	50	100.0	33	100.0	83	100.0

Table 16. ANA, ANCA andCD63 expression results

	CD63				Total	
	Positive		Negative			
ANA, ANCA:	N	%	N	%	N	%
ANA positive	5	9.3	2	6.9	7	8.4
ANA and ANCA positive	0	0.0	1	3.4	1	1.2
ANCA positive.	2	3.7	0	0.0	2	2.4
Normal	47	87.0	26	89.7	73	88.0
Total	54	100.0	29	100.0	83	100.0

Table 17. ANA, ANCA and CD203c expression results

	CD203c				Total	
	Positive		Negative			
ANA, ANCA:	N	%	N	%	N	%
ANA positive	4	8.2	3	8.8	7	8.4
ANA and ANCA positive	0	0.0	1	2.9	1	1.2
ANCA positive	1	2.0	1	2.9	2	2.4
Normal	44	89.8	29	85.3	73	88.0
Total	49	100.0	34	100.0	83	100.0

There was no significant correlation between ANA, ANCA and CAU markers: ASST ($p=0.092$, $\chi^2=6.442$, df=3), CD63 (0.380, $\chi^2=3.076$, df=3), CD203c ($p=0.667$, $\chi^2=1.565$, df=3). We propose examinate all patients for ANA as indicator of other autoimmune disoder.

6.5.3 Disease activity

Only 3 (2.6%) patients evaluated CU activity as „1“ point; 4 (3.4%) patients indicated „2“; 13 (11.2%) – „3“; 26 (22.4%) – „4“. The most patients evaluated their disease activity as „5“ -30 (25.9%) or „6“ – 40 (34.5%) points. Results are shown on Figure 7.

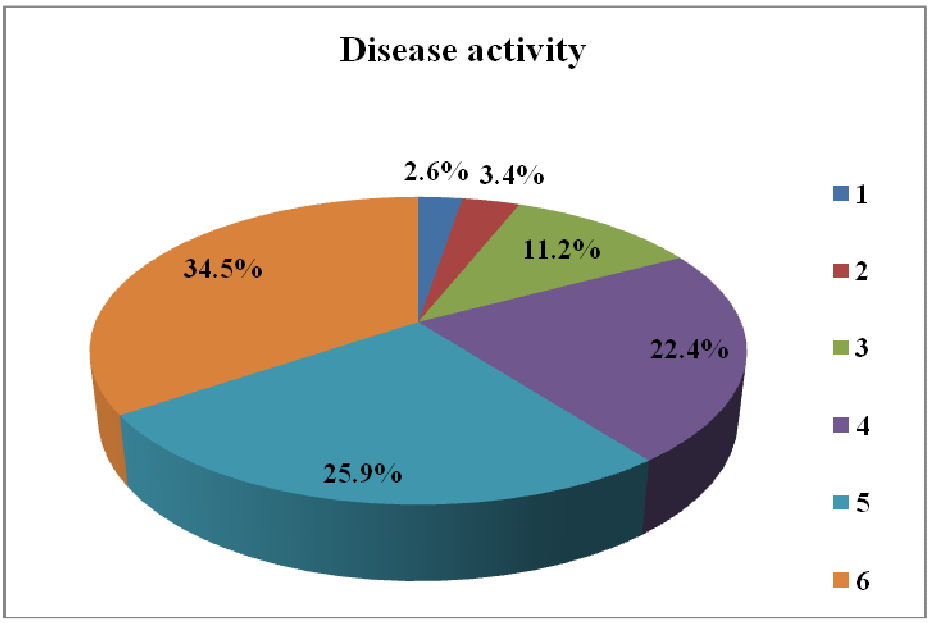


Figure 7. Disease activity

The relationsheep between disease activity and CAU markers is shown in Tables 18–20.

Table 18. Disease activity and ASST results

	ASST				Total	
	Positive		Negative			
Disease activity	N	%	N	%	N	%
1	3	3.8	0	0.0	3	2.6
2	4	5.1	0	0.0	4	3.4
3	8	10.3	5	13.2	13	11.2
4	14	17,9	12	31.6	26	22.4
5	21	26.9	9	23.7	30	25.9
6	28	35.9	12	31.6	40	34.5
Total	78	100.0	38	100.0	116	100.0

Table 19. Disease activity and CD63 expression results

	CD63				Total	
	Positive		Negative			
Disease activity	N	%	N	%	N	%
1	3	4.2	0	0.0	3	2.6
2	3	4.2	1	2.3	4	3.4
3	6	8.3	7	15.9	13	11.2
4	17	23.6	9	20.5	26	22.4
5	17	23.6	13	29.5	30	25.9
6	26	36.1	14	31.8	40	34.5
Total	72	100.0	44	100.0	116	100.0

Table 20. Disease activity and CD203c expression results

	CD203c				Total	
	Positive		Negative			
Disease activity	N	%	N	%	N	%
1	1	1.6	2	3.8	3	2.6
2	4	6.3	0	0.0	4	3.4
3	7	11.1	6	11.3	13	11.2
4	11	17.5	15	28.3	26	22.4
5	18	28.6	12	22.6	30	25.9
6	22	34.9	18	34.0	40	34.5
Total	63	100.0	53	100.0	116	100.0

We did‘t find correlation between disease activity and CAU markers: ASST ($p=0.310$, $\chi^2=5.962$, $df=5$), CD63 ($p=0.527$, $\chi^2=4.155$, $df=5$), CD203c ($p=0.325$, $\chi^2=5.807$, $df=5$).

6.5.4 The length of disease

The mean length of disease was 38 ± 70 months (min – 2 months, max – 40 years).
The relationsheep between length of disease and CAU markers is shown in Tables 21–23.

Table 21. Length of disease and ASST results

	ASST		Total
	Negative (N=78)	Positive (N=38)	
The lenght of disease (month)	Statistical value	Statistical value	Statistical value
Mean	41	33	38
SD	78	52	70
Median	9	6	8
Max	480	240	480
Min	2	3	2

Table 22. Length of disease and CD63 expression results

	CD63		Total
	Negative (N=72)	Positive (N=44)	
The lenght of disease (month)	Statistical value	Statistical value	Statistical value
Mean	34	45	38
SD	58	87	70
Median	8	7	8
Max	240	480	480
Min	2	2	2

Table 23. Length of disease and CD63 expression results

	CD203c		Total
	Negative (N=63)	Positive (N=53)	
The lenght of disease (month)	Statistical value	Statistical value	Statistical value
Mean	42	34	38
SD	80	57	70
Median	12	6	8
Max	480	240	480
Min	2	3	2

Mann-Whitney and Wilcoxon tests were used and we found no correlation between length of disease and CAU markers: ASST (p=0.470), CD63 (p=0.786), CD203c (p=0.153).

6.5.5 Angioedema

We found a very high incidence of angioedema in our patients – 89 (76.7%) (Figure 8). The most authors found angioedema association with CU in half of cases.

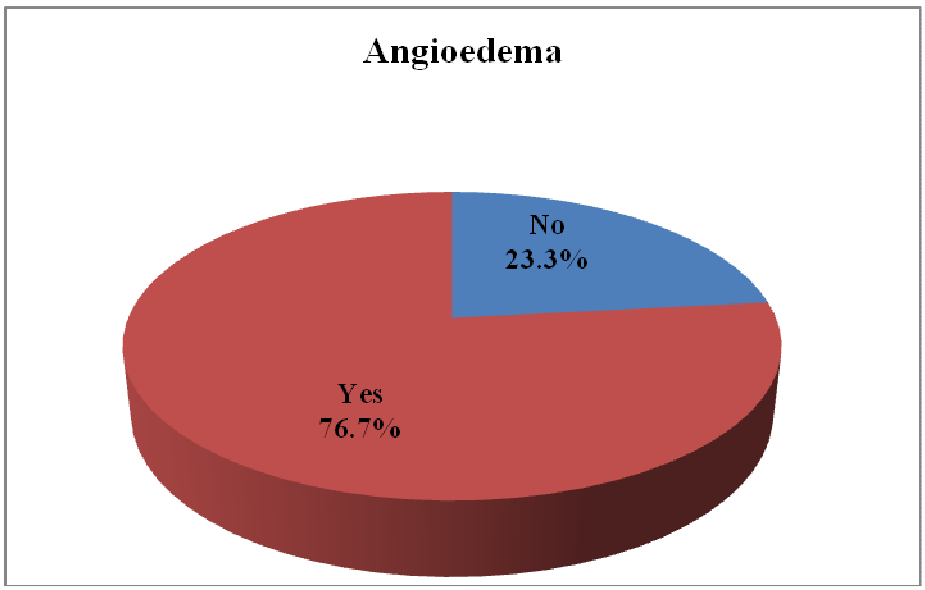


Figure 8. Angioedema incidence

The relationsheep between angioedema and LAD markers is shown in tables 24–26.

Table 24. Angioedema and ASST results

	ASST				Total	
	Positive		Negative			
Angioedema	N	%	N	%	N	%
No	20	25.6	7	18.4	27	23.3
Yes	58	74.4	31	81.6	89	76.7
Total	78	100.0	38	100.0	116	100.0

Table 25. Angioedema and CD63expression results

	CD63				Total	
	Positive		Negative			
Angioedema	N	%	N	%	N	%
No	20	27.8	7	15.9	27	23.3
Yes	52	72.2	37	84.1	89	76.7
Total	72	100.0	44	100.0	116	100.0

Table 26. Angioedema and CD203c expression results

	CD203c				Total	
	Positive		Negative			
Angioedema	N	%	N	%	N	%
No	17	27.0	10	18.9	27	23.3
Yes	46	73.0	43	81.1	89	76.7
Total	63	100.0	53	100.0	116	100.0

We did‘t find significant correlation between angioedema and LAD markers: ASST ($p=0.388$, $\chi^2=0.746$, $df=1$), CD63 ($p=0.142$,$\chi^2=2.154$, $df=1$), CD203c ($p=0.303$, $\chi^2=1.062$, $df=1$).

6.5.6 Hypersensitivity to NSAID‘s

12 (9.4%) patients reported hypersensitivity to different NSAID‘s (Figure 9). In all cases there were acute reactions appeared as urticaria/angioedema, one patient had symptoms of rhinitis and bronchospazm. The most patients were sensitive to one NSAID, only two indicated sensitivity to several chemically unrelated NSAID drugs.

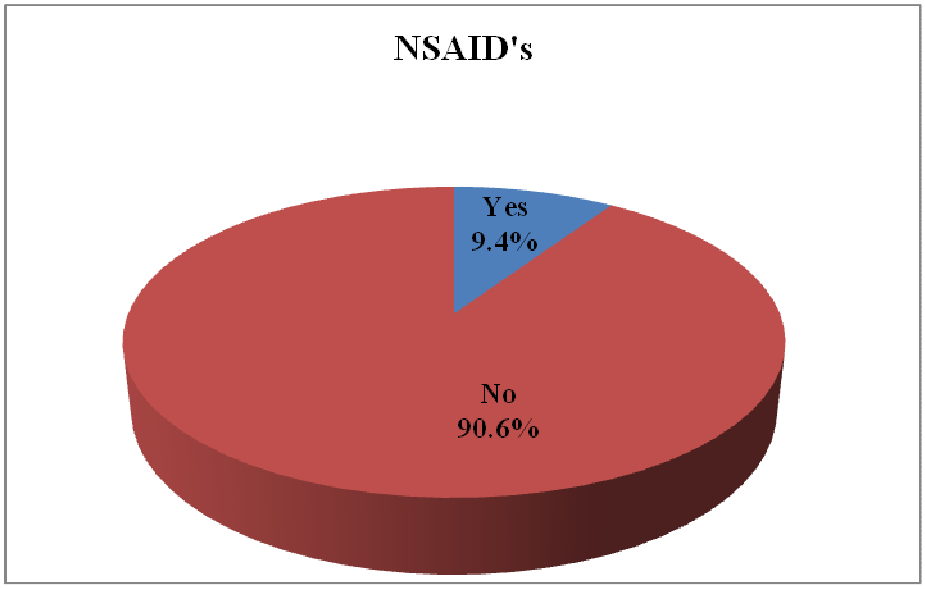


Figure 9. Hypersensitivity to NSAID‘s

The relationsheep between hypersensitivity to NSAID‘s and CAU markers is shown in tables 27–29.

Table 27. Hypersensitivity to NSAID‘s and ASST results

Hypersensitivity to NSAID‘s	ASST				Total	
	Positive		Negative			
	N	%	N	%	N	%
Yes	6	14.0	6	7.1	12	9.4
No	37	86.0	79	92.9	116	90.6
Total	43	100.0	85	100.0	128	100.0

Table 28. Hypersensitivity to NSAID‘s and CD63 results

Hypersensitivity to NSAID‘s	CD63				Total	
	Positive		Negative			
	N	%	N	%	N	%
Yes	6	12.8	6	7.4	12	9.4
No	41	87.2	75	92.6	116	90.6
Total	47	100.0	81	100.0	128	100.0

Table 29. Hypersensitivity to NSAID‘s and CD203c results

Hypersensitivity to NSAID‘s	CD203c				Total	
	Positive		Negative			
	N	%	N	%	N	%
Yes	7	12.1	5	7.1	12	9.4
No	51	87.9	65	92.9	116	90.6
Total	58	100.0	70	100.0	128	100.0

We did‘t find statistical correlation between hypersensitivity to NSAID‘s and CAU markers: ASST (p=0.206, χ^2=1.598, df=1), CD63 (p=0.316, χ^2=1.005, df=1), CD203c (p=0.341, χ^2=0.906, df=1), CD203c (p=0.206, χ^2=1.598, df=1).

6.5.7 Treatment efficacy

We found, that in 57 (44.5%) patients conventional treatment with antihistamines (AH) was uneffective (Figure 10).

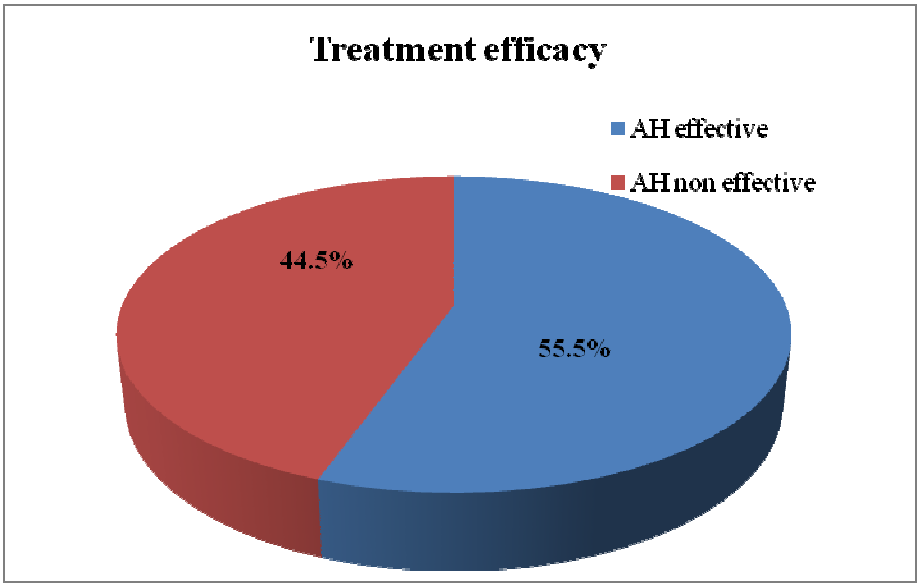


Figure 10. Treatment efficacy

The relationsheep between treatment efficacy and CAU markers is shown in tables 30–32.

Table 30. Treatment efficacy and ASST results

Treatment with AH	ASST				Total	
	Positive		Negative			
	N	%	N	%	N	%
Effective	18	41.9	53	62.4	71	55.5
Non effective	25	58.1	32	37.6	57	44.5
Total	43	100.0	85	100.0	128	100.0

Table 31. Treatment efficacy and CD63 results

Treatment with AH	CD63				Total	
	Positive		Negative			
	N	%	N	%	N	%
Effective	46	56.8	25	53.2	71	55.5
Non effective	35	43.2	22	46.8	57	44.5
Total	81	100.0	47	100.0	128	100.0

Table 32. Treatment efficacy and CD203c results

Treatment with AH	CD203c				Total	
	Positive		Negative			
	N	%	N	%	N	%
Effective	41	58.6	30	51.7	71	55.5
Non effective	29	41.4	28	48.3	57	44.5
Total	70	100.0	58	100.0	128	100.0

We found statistically significant correlation between treatment efficacy and ASST (**p=0.028, χ^2=4.855, df=1**), but there were no correlation with CD63 (p=0.693, χ^2=0.156, df=1) and CD203c p=0.438, χ^2=0.602, df=1). It showed that patients with histamine releasing factors in sera have more severe CU.

7 Conclusions

1. Sera from patients with CU significantly upregulate both CD63 and CD203c molekules on donor blood basophils. This result indicates the presence of basophil-activating factors (possibly autoantibodies) in patients sera.
2. Based on ROC curve analysis the estimated cut-off value of the basophil activation tests for autoimmune CU diagnosis was 10%.
3. As referred to the ASST the sensitivity and specifity of the in vitro assays were 55.8% and 72.9% for CD63; 55.8% and 60% for CD203c respectively.
4. There was a significant correlation between CD63 and ASST, and between CD63 and CD203c tests. However, no correlation between CD203c and ASST was found.
5. Better prognostic features of CD63 expression test in comparisson with CD203c, suggesting its potential usefulness for the diagnosis of autoimmune form of CU.
6. We didn‘t find significant correlation between ASST, CD63, CD203c expression and other autoimmunization markers (TPO, ANA, ANCA).
7. No correlation between ASST, CD63, CD203c expression and disease activity, length of the disease, angioedema, hypersensitivity to NSAID‘s was found.
8. Conventional treatment with antihistamines was less effective in patients with positive ASST compaired with negative ASST group.

8 List of publications

1. Chomiciene A, Blaziene A, Kowalski ML. Immunological mechanisms of chronic urticaria. Review of literature. Medicinos teorija ir praktika 2007; 2(13): 143–146.
2. Chomiciene A, Blaziene A, Jurgauskiene L. Serum induced CD63 upregulation on basophils as diagnostic tool in chronic autoimmune urticaria. Laboratorinė medicina 2008; 10, 4(40): 212–217.
3. Chomiciene A, Blaziene A. Chronic urticaria and autoreactivity. Medicinos teorija ir praktika 2008; 14(4): 322–327.
4. Chomiciene A, Blaziene A, Jurgauskiene L. Chronic urticaria and thyroid autoimmunity markers. Medicina (in press).

9 Reziumė

Autoreaktyvumo rodiklių diagnostinė reikšmė sergant lėtine dilgėline

Įvadas. Lėtinė dilgėlinė (LD) – liga, kuriai būdingi bėrimai pūkšlėmis su ar be angioedemos, spontaniškai atsirandantys odoje ir trunkantys ilgiau nei 6 savaites.

Nepaisant įvairių klinikinių ir laboratorinių tyrimų LD priežastis iki 75% atvejų lieka neaiški. Pacientų gyvenimo kokybė būna sutrikusi, simptomai turi įtakos jų fizinei ir emocinei būklei. Nustatyta, kad nuo 30 iki 50% LD atvejų galima paaiškinti autoimuniniu ligos išsivystymo mechanizmu. Todėl atsirado atskiras terminas – „lėtinė autoimuninė dilgėlinė“ (LAD). Pacientų, sergančių LAD, kraujyje randami dviejų tipų antikūnai: nukreipti prieš epitopus, ekspresuojamus didelio afiniškumo imunoglobulino E (IgE) receptoriaus (FcεRI) α-grandinėje (anti- FcεRIα) ir nukreipti prieš IgE (anti-IgE). Sergantiesiems LAD būdinga sunkesnė ligos eiga, jiems neveiksmingas įprastinis gydymas, dažnai diagnozuojamas autoimuninis tiroiditas.

LAD diagnozė nustatoma naudojant odos testą su autologiniu serumu (angl. *autologous serum skin test* – ASST) ir laboratorinius antikūnų tyrimus. ASST leidžia įtarti, bet nepatvirtina LAD diagnozės. Laboratoriniai Western blot analizės ir imunofermentinis metodai leidžia nustatyti antikūnus, bet neleidžia diferencijuoti, ar šie antikūnai yra funkciškai aktyvūs, ar ne. Tik histamino atpalaidavimo testas ir bazofilų aktyvacijos žymenų nustatymas tėkmės citometrijos būdu patvirtina sergančiojo LD kraujo serume esant funkciškai aktyvių antikūnų. Histamino atpalaidavimo testas yra sudėtingas, reikalaujantis daug laiko, įvairūs praktiniai ir techniniai šio testo trūkumai riboja jo naudojimą klinikinėje praktikoje. Daug vilčių teikiama bazofilų aktyvacijos testams naudojant tekmės citometrijos metodą. Tik šio šimtmečio pradžioje atsirado darbai apie bazofilų aktyvacijos nustatymą pagal CD63 ir CD203c žymenų ekspresiją donoro bazofilų paviršiuje ir jų panaudojimą LAD diagnostikai. Siekiant nustatyti kuris iš tyrimų turi geresnį jautrumą ir specifiškumą ir gali būti naudingesnis diagnozuoti LAD, reikėtų palyginti abiejų žymenų ekspresijos tyrimus.

LAD diagnozės nustatymas yra svarbus parinkti gydymo būdui bei įvertinti ligos prognozę.

Tyrimo tikslas. Nustatyti autoreaktyvumo rodiklių diagnostinę reikšmę ir jų tarpusavio ryšį pacientams, sergantiems LD.

Tyrimo uždaviniai:

1. Palyginti CD63 ir CD203c žymenų ekspresiją donoro bazofilų paviršiuje, paveikus juos sergančiųjų LD ir sveikųjų asmenų kraujo serumu.
2. Nustatyti bazofilų CD63 ir CD203c žymenų ekspresijos tyrimų ribinę vertę diagnozuojant LAD.
3. Nustatyti bazofilų CD63 ir CD203c žymenų ekspresijos tyrimų jautrumą ir specifiškumą bei jų reikšmę diagnozuojant LAD.
4. Įvertinti sergančiųjų LD bazofilų CD63 ir CD203c žymenų ekspresijos tyrimų ir ASST koreliaciją.

5. Įvertinti sergančiųjų LD bazofilų CD63 ir CD203c žymenų ekspresijos tyrimų, ASST ir kitų autoimunizacijos rodiklių (skydliaukės antikūnų prieš mikrosomų peroksidazę (ATPO), antikūnų prieš branduolių antigenus (ANA), antikūnų prieš neutrofilų citoplazmos antigenus (ANCA)) tarpusavio ryšį.
6. Įvertinti autoreaktyvumo rodiklių ryšį su LD aktyvumu, jos trukme, angioedemos pasireiškimu, jautrumu nesteroidiniams vaistams nuo uždegimo (NVNU) ir gydymo veiksmingumu.

Mokslinė darbo reikšmė ir naujumas:

1. Ištirta sergančiųjų LD bazofilų CD63 ir CD203c žymenų ekspresija ir palyginti tyrimų rezultatai.
2. Įvertinas bazofilų CD63 ir CD203c žymenų ekspresijos tyrimų jautrumas ir specifiškumas, naudojant skirtingas ribines vertes.
3. Nustatyta bazofilų CD63 ir CD203c žymenų ekspresijos tyrimų ir ASST koreliacija bei jų diagnostinė reikšmė diagnozuojant lėtinę autoimuninę dilgėlinę.
4. Įvertinta bazofilų CD63, CD203c žymenų ekspresijos tyrimų ir ASST koreliacija su kitais autoimunizacijos rodikliais.
5. Įvertintas autoreaktyvumo rodiklių ryšis su ligos aktyvumu, trukme, angioedemos pasireiškimu, jautrumu NVNU ir gydymo veiksmingumu.

Tiriamieji. Ištirti 128 pacientai, sergantys lėtine dilgėline: 26 vyrai ir 102 moterys. Pacientų amžiaus vidurkis – 43±13 (nuo 20 iki 78 metų). Kontrolinę grupę sudarė 30 sveikųjų asmenų: 15 vyrų ir 15 moterų, amžiaus vidurkis – 30±7 metai (nuo 22 iki 51 metų).

Tyrimo metodika. Visiems pacientams ir 10 kontrolinės grupės tiriamųjų atliktas ASST, suleidžiant į odą 50 µl tiriamojo asmens serumo ir tokį pat kiekį izotoninio 0,9% NaCl tirpalo neigiamai kontrolei. Mėginys vertintas po 30 ir 60 min. Mėginys vertintas kaip teigiamas, jeigu serumo sukelta papulė buvo ne mažiau kaip 1,5 mm didesnė už neigiamą kontrolę.

Visiems pacientams ir kontrolinės grupės tiriamiesiems atlikti bazofilų aktyvacijos testai nustatant CD63 ir CD203c žymenų ekspresiją atopinio donoro bazofilų paviršiuje.

CD203c žymens ekspresijos nustatymui naudota 40 µl tiriamojo serumo ir 200 µl heparinizuoto donoro kraujo. Ląstelės buvo žymimos naudojant PE prieš žmogaus CD45, PerCP prieš žmogaus CD203c, FITS prieš žmogaus IgE. CD63 žymens ekspresijos nustatymui naudota 100 µl tiriamojo kraujo ir 100 µl heparinizuoto donoro kraujo. Ląstelės buvo žymimos naudojant CD63FITS/CD123PE/Anti-HLA DR PerCP antikūnų kokteilį. Mėginiai analizuoti FACSCalibur tėkmės citometru (Becton Dickinson, JAV) naudojant CellQuest programinę įrangą. Bazofilų aktyvacija vertinta surenkant 1000 bazofilų.

Visų pacientų kraujyje pasitelkiant imunochemiliuminescentinį metodą nustatytas ATPO kiekis. TTH kiekis nustatytas tuo pačiu metodu visiems pacientams, kuriems ATPO kiekis buvo padidėjęs ir 48, kurie turėjo normalų ATPO kiekį.

83 pacientams netiesioginės imunofluorescentinės analizės metodu ištirti ANA ir ANCA antikūnai.

116 pacientų atsakė į sudarytos anketos klausimus (21 klausimas) pagal EAACI/GA²LEN/EDF sudarytą klausmyną sergantiesiems LD. Šiame darbe pateikiame tik LD aktyvumo, trukmės, angioedemos, jautrumo nesteroidiniams vaistams nuo uždegimo (NVNU), įprastinio gydymo veiksmingumo ryšį su LAD rodikliais (ASST, CD63 ir CD203c žymenų ekspresija).

Statistinė analizė atlikta naudojant statistinį paketą SPSS 15.0. Kiekybinių kintamųjų koreliacijai vertinti skaičiavome Spearmann'o koreliacijos koeficientą. Vidurkių lygybės hipotezei tikrinti buvo naudojamas Wilcoxon-Mann-Whitney testas. Požymių analizės priklausomybės vertinimui buvo naudojamas chi kvadrato (χ^2) testas. Tikrinant statistines hipotezes, buvo pasirinktas 0,05 reikšmingumo lygmuo.

Rezultatai. Pacientų CD63 žymens ekspresijos tyrimo rezultatai (vidurkis±SD): 10,8%±10,9%; CD203c: 11,8%±12,4%. Kontrolinėje grupėje CD63: 3,10%±1,5%; CD203c: 1,84%±0,5%. Mann-Whitney testas parodė, kad bazofilų aktyvacijos testas tiek CD63, tiek CD203c žymens ekspresija reikšmingai skiriasi pacientų ir kontrolinės grupės asmenų ($p<0,001$).

Apskaičiavome septynias dažniausiai naudojamas ribines bazofilų aktyvacijos tyrimų vertes: kontrolinės grupės vidutinė reikšmė +2 standartiniai nuokrypiai; kontrolinės grupės vidutinė reikšmė +3 standartiniai nuokrypiai; 5%; 10%; 15%; 2x neigiama kontrolė; 95 kontrolinės grupės procentilis. Panaudojome Galen-Gambino formulę testų jautrumui ir specifiškumui įvertinti, ASST buvo naudojamas kaip "auksinis standartas", pagal kurį vertinome CD63 ir CD203c žymenų ekspresijos tyrimų rezultatus. Papildomai optimali ribinė vertė buvo įvertinta naudojant gautų duomenų efektyvumą charakterizuojančias kreives (angl. *receiver operator characteristic* – ROC) bei apskaičiuojant po jomis esantį plotą. Kadangi CD203c plotas po kreive yra artimas 0,5 ir p reikšmė >0,05, padarėme išvadą, kad šis tyrimas nepasižymi geromis prognostinėmis savybėmis palyginus su CD63 žymens ekspresijos tyrimu. CD63 testo klasifikacijos tikslumui vertinti naudojome Youdeno indeksą ir bendrą tikslumo rodiklį, paklaidai vertinti – bendros klasifikacijos paklaidos rodiklius (angl. *total misclassification error* – TME). Remiantis gautais duomenimis ir ROC kreivės analize, pasirinkome optimaliausią ribinę vetę – 10%. Visiems skaičiavimams toliau naudojome šią vertę. Didžiausias CD63 žymens ekspresijos tyrimo jautrumas – 55,8%, specifiškumas – 72,9%; CD203c jautrumas – 55,8%, specifiškumas – 60,0%. Teigiamų CD63 žymens tyrimų skaičius – 36,7%; CD203c – 45,3%.

Sergančiųjų LD ASST buvo teigiamas 43 (33,6%). Kontrolinėje grupėje ASST visais atvejais buvo neigiamas. Įvertinus ryšį tarp ASST ir CD63, nustatyta patikima koreliacija tarp šių tyrimų ($r=0,282$, $p=0,001$). Tačiau gautas priešingas rezultatas įvertinus ASST ir CD203c ryšį ($r=0,150$, $p=0,091$), taigi ASST ir CD203c nekoreliuoja tarpusavyje. Patikrinome CD63 ir CD203c žymenų ekspresijos testų nepriklausomumą pagal Chi-Square kriterijų ir nustatėme, kad šių testų rezultatai yra priklausomi tarpusavyje ($p<0,001$).

32 (25,0%) pacientų ATPO buvo padidėjęs. Padidėjęs TTH, rodantis skydliaukės hipofunkciją, nustatytas tik pacientų, kuriems buvo padidėję ATPO grupėje (12,5% pacientų). Skydliaukės hiperfunkcija (sumažėjęs TTH) nustatyta abiėjose (ir turėjusių padidėjusį ir normalų ATPO kiekį) grupėse – 15,6% ir 8,3% atitinkamai. Statistiškai reikšmingo ryšio tarp ATPO ir LAD rodiklių (ASST, CD63, CD203c tyrimų) negavome, nustatytas reikšmingumo lygmuo visais atvejais mažesnis nei 0,05.

ANA buvo teigiamas 7 (8,4%) pacientų, ANCA teigiamas – 2 (2,4%) pacientų, abu antikūnai rasti tik 1 (1,2%) pacientui. Negavome patikimos ANA, ANCA koreliacijos su LAD rodiklias ($p<0,05$).

Ligos aktyvumas buvo įvertintas „1" – 3 (2,6%), "2" – 4 (3,4%), "3"– 13 (11,2%), "4"– 26 (22,4%), "5"– 30 (25,9%), "6"– 40 (34,5%) pacientų. Ligos aktyvumas nebuvo rekšmingai susijęs su LAD rodikliais ($p<0,05$).

Ligos trukmės vidurkis – 38 mėn.±70 mėn. (nuo 2 mėn. iki 40 metų). Patikimos korelacijos tarp ligos trukmės ir LAD rodiklių negavome ($p<0,05$).

Angioedemos epizodai kartu su LD pasireiškė net 89 (76,7%) pacientų. Patikimo ryšio tarp angioedemos pasireiškimo ir LAD rodiklių neradome ($p<0,05$).

Jautrumą NVNU nurodė 12 (9,4%) pacientų. Visi pacientai nurodė greito tipo reakcijas į NVNU, dauguma pacientų buvo jautrūs tik vienam preparatui ir tik keli nurodė jautrumą keliems NVNU. Jautrumas NVNU nebuvo reikšmingai susijęs su LAD rodikliais.

Gydymas vien antihistamininiais preparatais net 57 (44,5%) pacientų buvo neveiksmingas. Gavome statistiškai reikšmingą gydymo antihistamininias preparatais veiksmingumo skirtumą tarp pacientų grupių, kuriems ASST buvo teigiamas ir neigiamas. Tai reiškia, kad pacientams, kuriems ASST buvo teigiamas, dažniau teko skirti gydymą alternatyviais preparatais, taikyti imunoterapiją.

Išvados:

1. Bazofilų aktyvacijos žymenų ekspresija (CD203c ir CD63) reikšmingai didesnė sergančiųjų LD, palyginus su sveikais asmenimis. Tai leidžia įtarti, kad sergančiųjų lėtine dilgėline kraujo serume yra histaminą atpalaiduojančių faktorių (galimai funkciškai aktyvių autoantikūnų).
2. Optimali CD63 ir CD203c žymenų ekspresijos tyrimų ribinė vertė yra 10%.
3. Naudojant 10% ribinę vertę autoimuninei dilgėlinės kilmei nustatyti, bazofilų CD63 žymens ekspresijos tyrimo jautrumas buvo 55,8%, specifiškumas – 72,9%; o CD203c tyrimo jautrumas – 55,8%, specifiškumas – 60,0%.
4. Bazofilų CD63 žymens ekspresijos tyrimo rezultatai patikimai koreliuoja su ASST ir su CD203c žymens ekspresijos testo rezultatais. Patikimos CD203c žymens koreliacijos su ASST neradome.
5. Bazofilų CD63 žymens ekspresijos tyrimas, nustatant autoimuninę dilgėlinės kilmę, pasižymi geresnėmis prognostinėmis savybėmis, palyginti su CD203c žymens ekspresijos tyrimu, todėl gali būti naudojamas LAD diagnozuoti.
6. Patikimos koreliacijos tarp ASST, bazofilų CD63, CD203c žymenų ekspresijos tyrimų ir kitų autoimunizacijos rodiklių (ATPO, ANA, ANCA) nenustatyta.
7. Reikšmingos sąsajos tarp ASST, bazofilų CD63, CD203c žymenų ekspresijos tyrimų ir ligos aktyvumo, jos trukmės, angioedemos pasireiškimo, padidėjusio jautrumo NVNU nerasta.
8. Nusatėme, kad įprastinis gydymas antihistamininiais preparatais yra mažiau veiksmingas pacientams, kuriems ASST teigiamas.

10 Curriculum vitae

Anželika Chomičienė was born in Vilnius, Lithuania on August 04, 1970

Working place
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Medical education
1988–1994 – Medical faculty of Vilnius University
1994–1995 – primary residency (Vilnius University Hospital)
1995–1997 – residency in internal medicine (Vilnius University Hospital)
1997–1999 -–fellowship in allergy (Vilnius University Hospital)
2005 – 2009 – doctoral studies in Vilnius University

Postgraduate courses
1999 08 – Summer school of EAACI in Poland
2000 06 – Summer course on immunology of asthma in Italy
2000 09 – Summer school of EAACI in Italy
2001 08 – Summer school of EAACI in Ucraine
2003 08 – Summer school of EAACI in Lithuania
2005 02 – 03 and 2006 03 – Scientific traineeship in Department of Clinical Immunology and Allergy, Medical University of Lodz (Poland)
2008 01– CHOP seminar in Salzburg "Pediatric Allergy and Immunology"

Professional societes
Member of Lithuanian Society of Allergology and Clinical Immunology (since 1997) and member of EAACI (since 1998).

Main field of scientific interest
Chronic urticaria, cold urticaria

Approbation

Poster presentations:

1. Chomiciene A, DuBuske LM, Blaziene A. Association of chronic idiophatic urticaria with autoimmunity and IgE levels. American Academy of Allergy Asthma and Immunology (AAAAI) 2007 Annual meeting, San Diego.
2. Chomiciene A, Blaziene A, Kvedariene V, Grigaitiene J. Chronic autoimmune urticaria. EAACI 2007 XXVI Congress, Goteborg.
3. Blaziene A, Chomiciene A, Tursaite J, DuBuske LM. Autoimmune disease and chronic urticaria in adult patients in Lithuania. AAAAI 2008 Annual meeting, Philadelphia.

4. Chomiciene A, Blaziene A, Grigaitiene J, Ilginiene J. Thyroid autoimmunity in chronic urticaria. EAACI 2008 XXVII Congress, Barselona.
5. Ilginine J, Chomiciene A, Blaziene A. Chronic urticaria: how do patients assess it severity, treatment's efficacy and quality of life? EAACI 2008 XXVII Congress, Barselona.
6. Chomiciene A, Blaziene A, Jurgauskiene L, Dubuke LM. Increased basophil CD63 and CD203c expression versus autologous serum skin tests in chronic urticaria patients. American College of Allergy, Asthma & Immunology (ACAAI) 2008 Annual meeting, Seattle.
7. Chomiciene A, Jurgauskiene L, Dubuke LM, Blaziene A. Identification of autoimmune urticaria in chronic urticaria patients by autologous serum skin test versus basophil activation tests. AAAAI 2009 Annual meeting, Washington.

Oral presentations:

1. 2005.05.25 – Urticaria: allergic or nonallergic disease? First Baltic Allergy Congress in Vilnius.
2. 2008.05.12 – The role of autoreactivity in chronic autoimmune urticaria. Vilnius University Medical Faculty, Lithuanian Society of Allergology and Clinical Immunology, ACAAI scientific-practical conference „Allergic diseases: from family doctor to specialist", Vilnius.