VILNIUS UNIVERSITY
STATE RESEARCH INSTITUTE
CENTRE FOR INNOVATIVE MEDICINE

Gintaras Sūdžius

CHANGES OF THE IMMUNE SYSTEM IN THE PATHOGENESIS OF PRIMARY SJÖGREN'S SYNDROME

Summary of doctoral thesis
Biomedical sciences, biology (01B);
Immunology, serology, transplantation (B500)

Vilnius, 2013

This study was carried out in 2008-2012 at the Institute of Immunology of Vilnius University and, after reorganization, at the State Research Institute Center for Innovative Medicine

Scientific Supervisor:

Dr. Almantas Šiaurys (State Research Institute Center for Innovative Medicine, biomedical sciences, biology – 01B, immunology, serology, transplantation – B500).

The dissertation is defended at the Research Board for Biology of Vilnius University:

Chairman:

dr. Mykolas Mauricas (State Research Institute Center for Innovative Medicine, biomedical sciences, biology – 01B, immunology, serology, transplantation – B500).

Members:

prof. habil. dr. Aniolas Sruoga (Vytautas Magnus University, biomedical sciences, biology – 01B, immunology, serology, transplantation – B500)

doc. dr. Irutė Girkontaitė (State Research Institute Center for Innovative Medicine, biomedical sciences, biology – 01B, immunology, serology, transplantation – B500).

dr. Vita Pašukonienė (Institute of Oncology, Vilnius University, biomedical sciences, biology – 01B, immunology, serology, transplantation – B500)

dr. Augustas Pivoriūnas (State Research Institute Center for Innovative Medicine, biomedical sciences, medicine – 06B, histology, chemistry of celės, histological chemistry, tissue culture – B210)

Opponents

doc. dr. Astra Vitkauskienė (Lithuanian University of Health Sciences, biomedical sciences, medicine – 06B, immunology, serology, transplantation – B500)

prof. dr. Genė Biziulevičienė (Vilnius University, biomedical sciences, biology – 01B, immunology, serology, transplantation – B500)

Defence of the doctoral dissertation will take place at the open meeting held by the Research Board for Biology on 19 of December 2013 in the hall of State Research Institute Center for Innovative Medicine. Address: Molėtų pl. 29, Vilnius, Lithuania.

The summary of the dissertation was sent on 15 of November 2013.

The dissertation is available at the Library of State Research Institute Center for Innovative Medicine and Vilnius University Library.

VILNIAUS UNIVERSITETAS
VALSTYBINIS MOKSLINIŲ TYRIMŲ INSTITUTAS
INOVATYVIOS MEDICINOS CENTRAS

Gintaras Sūdžius

IMUNINĖS SISTEMOS POKYČIAI PIRMINIO SJÖGRENO SINDROMO PATOGENEZĖJE

Daktaro disertacijos santrauka
Biomedicinos mokslai, biologija (01B),
Imunologija, serologija, transplantacija (B 500)

Vilnius, 2013

Disertacija rengta 2008-2012 m. Vilniaus universiteto Imunologijos institute ir po reorganizacijos Valstybiniame mokslinių tyrimų institute Inovatyvios medicinos centre.

Mokslinis vadovas:

dr. Almantas Šiaurys (Valstybinis mokslinių tyrimų institutas Inovatyvios medicinos centras, biomedicinos mokslai, biologija – 01B, imunologija, serologija, transplantacija – B500).

Disertacija ginama Vilniaus universiteto Biologijos krypties taryboje:

Pirmininkas:

dr. Mykolas Mauricas (Valstybinis mokslinių tyrimų institutas Inovatyvios medicinos centras, biomedicinos mokslai, biologija – 01B, imunologija, serologija, transplantacija – B500).

Nariai:

prof. habil. dr. Aniolas Sruoga (Vytauto Didžiojo universitetas, biomedicinos mokslai, biologija – 01B, imunologija, serologija, transplantacija – B500)

doc. dr. Irutė Girkontaitė (Valstybinis mokslinių tyrimų institutas Inovatyvios medicinos centras, biomedicinos mokslai, biologija – 01B, imunologija, serologija, transplantacija – B500).

dr. Vita Pašukonienė (Vilniaus universiteto Onkologijos institutas, biomedicinos mokslai, biologija – 01B, imunologija, serologija, transplantacija – B500)

dr. Augustas Pivoriūnas (Valstybinis mokslinių tyrimų institutas Inovatyvios medicinos centras, biomedicinos mokslai, medicina – 06B, histologija, ląstelių chemija, histologinė chemija, audinių kultūra – B210)

Oponentai:

doc. dr. Astra Vitkauskienė (Lietuvos sveikatos mokslų universitetas, biomedicinos mokslai, medicina – 06B, imunologija, serologija, transplantacija – B500)

prof. dr. Genė Biziulevičienė (Vilniaus universitetas, biomedicinos mokslai, biologija – 01B, imunologija, serologija, transplantacija – B500)

Disertacija bus ginama viešame Biologijos mokslo krypties tarybos posėdyje 2013 m. gruodžio 19d. Valstybinio mokslinių tyrimų instituto posėdžių salėje. Adresas: Molėtų pl. 29, Vilnius, Lietuva.

Disertacijos santrauka išsiuntinėta 2013 m. lapkričio 15d.

Disertaciją galima peržiūrėti Valstybinio mokslinių tyrimų instituto Inovatyvios medicinos centro ir Vilniaus universiteto bibliotekose.

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disorder of the exocrine glands with still unknown aetiology. It is typically associated with focal lymphocytic infiltrates of glandular tissues and autoantibody responses against the Ro(SSA) and La(SSB) ribonucleoproteins. The hallmark symptom of Sjögren's syndrome is a generalized dryness, typically including xerostomia (dry mouth) and keratoconjunctivitis sicca (dry eyes). Sjögren's syndrome may cause skin, nose and vaginal dryness and may also affect other organs of the body (including the kidneys, blood vessels, lungs, liver, pancreas, a peripheral nervous system and brain). Patients with primary Sjögren's syndrome (pSS), as compared with the general population, have 44 times higher risk of non-Hodgkin lymphoma development. Patients with severe cases are much more likely to develop lymphomas than patients with mild or moderate cases. Sjögren's syndrome can exist as a disorder in its own right (primary Sjögren's syndrome) or may develop years after the onset of an associated rheumatic disorder, most commonly such as rheumatoid arthritis, systemic lupus erythematosus etc. (secondary Sjögren's syndrome). In almost one-third of patients disease involves various extra-glandular sites; thus typically the primary Sjögren's syndrome patients can be divided into two groups: patients with damaged just tear and salivary glands (glandular manifestations), and patients with also systematically damaged other organs and tissues (extra-glandular manifestations). Primary Sjögren's syndrome can occur in patients of all ages, but the most commonly in the fourth to fifth decade of women's life. Sick women and men ratio is 9:1. Few decades ago, Sjögren's syndrome was a rarely determined disease; however, the improvements in the diagnosis and the adoption of the uniform diagnostic criteria (American-European Consensus Criteria) increased diagnosed cases by almost 10 times. Nowadays, it is currently one of the most common autoimmune diseases. The overall prevalence of Sjögren's Syndrome is estimated to be between 0.05 and 0.48% of the total population. Previously, Sjögren's syndrome was often undiagnosed by attributing the observed symptoms, similar to the Sjögren's syndrome, to other diseases. Often in case of such mimicry and poorly expressed symptoms of the disease, Sjögren's syndrome is diagnosed in the first 6-10 years after the onset of the first symptoms and the

onset of the disease. Sjögren's syndrome is a chronic autoimmune disease with still unclear etiology. As an autoimmune disease, susceptibility to Sjögren's syndrome is greatly influenced by the heredity (genetic factor), hormones (e.g., estrogen, as is considered that 90 percent sufferers are women), and exogenous factors (viral or bacterial infections). Disease occurs during a specific, self-perpetuating immune system-mediated loss of exocrine gland function, which begins after the infiltration of lymphocytes and plasma cells in the salivary gland ducts and glandular tissues. Despite intensive research of the immune system, the model of the Sjögren's syndrome pathogenesis is not completely clear. There are many studies done in the investigation of minor salivary gland biopsy material (lymphocyte populations in lymphocytic infiltrates, humoral factors and their interaction with the immune system cells). However, even the published results are often controversial. This might be explained by the inconsistency of research, different research models/systems and also demographic differences of patients (different sets of genes are specific to a certain population). Less attention by the researchers is paid to the peripheral blood immune system cells, and these results are often also controversial. Lymphopenia is a common symptom found in the pSS patients. Numerous studies are performed in order to determine the causes of lymphopenia, but there is a lack of detailed studies to reveal which cell population counts increase or decrease. Scarce studies are done to associate the changes in the immune cell population and the expression of humoral factors in the peripheral blood of pSS patients. Peripheral blood is a convenient object for the research, thus it is very important to screen blood and serum for new biomarkers. However, it is more important now to use the biologic therapies for the treatment of autoimmune diseases.

The aim of dissertation work is to investigate the changes of the components of the systemic immune response in the peripheral blood of patients with primary Sjögren's syndrome (pSS) with different manifestations of the disease.

The objectives of this work were as followed:

1. Identification and quantitative analysis of B, NK and T lymphocytes in the peripheral blood of pSS patients.
2. Quantitative analysis of effector and regulatory $CD4^+$ (by IL17A and CD25Foxp3 markers) and $CD8^+$ (by CD57 and CD27 markers) T cell populations in the peripheral blood of patients with primary Sjögren's syndrome.
3. Evaluation of the relative changes of T helper (Th1, Th2 and Th17 Th17/Th1-like) cell populations in the peripheral blood of pSS patients.
4. Investigation of IgG, κ and λ FLC, BAFF and fragment C4d of the complement component C4 serum levels, also focusing on their associations with the cell immunity components in patients with pSS.
5. Investigation of IL-27 and IL-35 levels in serum of pSS patients.

Scientific novelty

In this study, a comprehensive analysis of B, NK and T cell populations in the peripheral blood of pSS patients was performed. It is the first detailed study, as most of studies are concerned with the research of immune cell products, such as cytokines and other factors in serum and glandular tissue biopsy. For the first time the expression of CD57 and CD27 markers on $CD8^+$ T cell population was analyzed and Th17/Th1-like cells in the peripheral blood of pSS patients were identified. In this work, were identified new potential immunological biomarkers ($CD4^+IL17A^+$, $CD8^iCD57^-CD27^+$ and Th17/Th1-like lymphocytes) in the peripheral blood of pSS patients. An imbalance in the distribution of T helper cell population was revealed. It can be explained by the decrease of Th2 population and increase of Th17/Th1-like lymphocyte population. The levels of IL-27 and IL-35 in sera of pSS patients were measured (not in the model system) for the first time as well. The increased levels of these interleukins in the sera of pSS patients show that they are implicated in pSS pathogenesis. Investigated serum levels of IgG, κ and λ FLC, BAFF and complement C4 fragment C4d, revealed their association with the cell immunity components in patients with pSS.

The defensive statements

- Lymphopenia, typically detected in patients with primary Sjögren's syndrome, is caused by a decrease of the absolute counts of NK and specific T cell ($CD4^+$ and $CD8^+$) populations and their subpopulations ($CD4^+IL17A^+$, $CD8^iCD57^-CD27^+$) in peripheral blood.

- There is an imbalance in the distribution of T helper cell populations. The reason for this is the decrease tendency of Th2 population and the significant increase of Th17/Th1-like lymphocyte percentage.

- Primary Sjögren's syndrome patients with extra-glandular manifestations have higher imbalance of B cell homeostasis, than patients with only glandular manifestations. This is caused by an imbalance in the expression of the humoral factors.

- IL-27 and IL-35 are implicated in the pSS pathogenesis.

Materials and methods

Patients. In total, 106 patients with pSS and 82 healthy controls were recruited at the State Research Institute Center for Innovative Medicine for this study. The study was performed in 4 steps. Serum levels of IL-27 and IL-35 were measured for 50 pSS patients and 50 controls. Qualitative analysis of T helper cells was performed for 37 pSS patients and 26 controls. Quantitative analysis of B, NK and T lymphocyte populations was performed for 53 pSS patients (the patients were divided into 2 groups - 30 patients without (1pSS group) and 23 patients with extra-glandular involvements (2pSS group)) and 32 controls. Serum levels of BAFF, κ and λ FLC and C4d were tested in 58 pSS patients (35 1pSS, 23 2pSS patients) and 19 controls while the amount of IgG - for 50 pSS patients (29 1pSS, 21 2pSS patients) and 24 controls. All patients underwent an extensive medical examination. Following the initial evaluation by a rheumatologist, each patient was referred to SS specialists for a review of his/her medical history, an oral and an ocular examination, Shirmer's I test, unstimulated salivary flow rate and a labial gland biopsy. Also, all patients underwent serological evaluations, which included tests for the presence of antibodies against Ro/SSA and La/SSB. All patients were diagnosed according to American–European Consensus Group criteria. Informed and written consent was obtained from all patients who participated in this study. The study has been approved by the Lithuanian Bioethics Committee (No. 158200-03-299-73).

Quantitative analysis of B, NK and T lymphocyte populations. Blood samples were collected from heparinized venous blood. Absolute counts of white blood cells were determined with a haemocytometer and used for calculation of absolute counts of lymphocyte populations (numbers of cells/μL peripheral blood). For cell surface staining were used the following monoclonal antibodies (mAbs): anti-CD3 FITC (Exbio, Czech); anti-CD4 PerCP (BD, USA); anti-CD8-PerCP (BD); anti-CD-16+56-PE (Exbio, Czech); anti-CD-19 PerCP (Exbio, Czech Republic) Mouse anti-IgG1-FITC, IgG1- PerCP and IgG2a-PE (BD, USA) were used for isotype control staining. Staining was performed at room temperature for 30 min., followed by red blood cell lysis using Pharm Lyse (BD) lysing solution for 15 min. at room temperature in the dark. Then, the leucocytes were

centrifuged (at 500 g for 10 min.), washed two times with CellWash (BD) and resuspended in FBS (BD). Samples were examined immediately after staining without fixation. For the evaluation of intracellular cytokines of CD4+ T cells, 1 ml of whole heparinized blood was diluted 1:2 in RPMI-1640 supplemented with 80 mg/l gentamycin and 2 nM glutamine. Cells were stimulated using 50 ng/ml phorbol–myristate–acetate (Sigma Aldrich, St Louis, MO, USA) and 1 ng/ml ionomycin (Sigma Aldrich) in the presence of 0,7 μl/ml monensin (GolgiStop (BD, USA)) for 4.5h at 37°C in an atmosphere containing 5% CO2. Unstimulated cells served as controls. Following stimulation, cells were stained for CD4 for 30 min at room temperature. Cell staining was followed by red blood cell lysis using Pharm Lyse (BD) lysing solution for 15 min. at room temperature in the dark. Leucocytes were then centrifuged (at 500 g for 10 min.) and washed two times with CellWash (BD). Then, cells were fixed and permeabilized with Cytofix/Cytoperm (BD) solution for 20min, washed two times with Perm/Wash solution and incubated further for 30 min in the dark with the specific mAbs anti-IL17A APC and isotypic control anti-IgG1 APC. Following the incubation with mAbs, the cells were washed two times with Perm/Wash solution and resuspended in FBS (BD, USA).

Qualitative analysis of T helper cells. For qualitative analysis of T helper cells the purification and stimulation of peripheral blood mononuclear cells (PBMCs) was performed. PBMCs were isolated from heparinized peripheral venous blood by ficoll (Ficoll-Paque PLUS, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) density gradient centrifugation, then were washed with phosphate-buffered saline and resuspended in RPMI 1640 media supplemented with 10% fetal calf serum and 1% glutamine. Cells at the concentration of 1-10 x10^6 cells/ml were stimulated for 4.5h with phorbol-12-myristate-13-acetate (50 ng/ml; AppliChem GmbH, Darmstadt, Germany) and ionomycin (1 μg/ml; AppliChem GmbH, Darmstadt, Germany) in the presence of GolgiStop (BD Biosciences, San Diego, CA, USA) at 37° C in 5% CO_2. Stimulated PBMCs were fixed and permeabilized with Fixation/Permeabilization Kit buffers (BD Cytofix and BD Perm/Wash, BD Biosciences, San Diego, CA, USA).Then, then cells were stained with Human Th1/Th2/Th17 Phenotyping Cocktail (BD Pharmingen, San Diego, CA, USA) containing:

antihuman CD4 PerCP-CY5.5, antihuman IL-17A PE, antihuman IFN-γ FITC, antihuman IL-4 APC. For the appropriate negative control the cells were stained with Three-color Fluorescent Ig Isotype Coctail (BD Pharmingen, San Diego, CA, USA) containing: antihuman CD4 PerCP-CY5.5 and isotype controls FITC-Mouse IgG1, PE-Mouse IgG1 and APC-Rat IgG1. Th1 cells were defined as $CD4^+$ IFN-γ^+, Th17 cells were defined as $CD4^+$ IL-$17A^+$, Th2 cells were defined as $CD4^+$ IL-4^+ and Th17/Th1-like cells were defined as $CD4^+$ IL-$17A^+$ IFN-γ^+.

Flow cytometrical analysis. Four-color flow cytometry was performed on FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA) calibrated with CaliBRITE beads (BD Biosciences, San Jose, CA, USA) using CELL-Quest software (BD Biosciences, San Jose, CA, USA). Lymphocytes, granulocytes and monocytes were gated and separated based on their morphological properties. Data for each sample were acquired until 100,000 lymphocytes were analyzed.

BAFF, κ and λ FLC, C4d and IgG serum level determination. Serum samples were analyzed by commercial ELISA kits: MicroVue C4d fragment EIA kit (Quidel Corporation, USA); Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA kit (BioVendor-Laboratorni medicina a.s., Czech Republic); BAFF, Soluble (human) ELISA Kit (hypersensitive) (AdipoGen, Switzerland) and Human Immunoglobulin G ELISA Kit (Wuhan EIAab Science Co., Ltd, China).

Blood samples, collected in non-additive silicone coated tubes, were allowed to clot at room temperature for 30 min before centrifugation (3000 rpm, 4^0C for 10 min.). After centrifugation, the serum was removed and stored at -80^0 C until analyzed. Serum dilutions and enzyme-linked immunoassay were carried out in a strict accordance with the manufacturer's instructions and recommendations. The results were evaluated using the spectrophotometer (BioTek Instruments, USA). The concentrations of analytes in ELISA assays were quantified using standard curves. A regression analysis was performed to derive an equation that was used to predict the concentration of the unknown samples with Gen5 Microplate Data Collection & Analysis Software (BioTek Instruments, USA).

The expected values in serum from healthy donors were indicated in the kit operating instructions:

C4d fragment level range in plasma and serum is 1.2 – 8.0 μg/ml (mean 4.6 μg/ml), BAFF - from 0.5 to >5 ng/ml, κ free light chains -11.5 ± 6.72 mg/l (mean ±2xSD), λ free light chains -17.39 ± 13.68 mg/l (mean ±2xSD); κ/λ ratio – 0.707 ± 0.466 (mean ±2xSD) and IgG – 4-16 mg/ml (from literature).

Statistics. Statistical differences were analyzed with the Mann-Whitney U test and correlations were assessed by Spearman's rank test using standard program GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). *P* values less than 0.05 were considered significant.

Results and discussion.

Quantitative analysis of B, NK and T lymphocyte populations. The evaluation of peripheral blood lymphocytes with a common to all T lymphocytes marker CD3, showed a significant decrease of absolute T -cell count in the peripheral blood of patients with primary Sjögren's syndrome, patient groups without extra-glandular manifestations ($\mathbf{p}_{1pSS\text{-}K}$=0.0042) and extra-glandular manifestations ($\mathbf{p}_{2pSS\text{-}K}$=0.0004) in comparison to the control group results. No significant difference was observed between 1pSS and 2pSS groups ($\mathbf{p}_{1pSS\text{-}2pSS}$=0.337) (Fig. 1.). The decrease of T cell absolute count in pSS patients may be associated with cell migration to inflammatory sites and/or more intense apoptosis of the peripheral T-cells. These assumptions belong to Ichikawa and colleagues; they determined lymphopenia in pSS patients and also found the increased Fas+ cell counts in the patient $CD4^+$ and $CD8^+$ cell populations. Ogawa and colleagues also found an increased apoptosis of peripheral blood T –cells in pSS patients after 48 hours of cultivation *in vitro*. The authors suggest, that this is due to the increased apoptosis promoters Bax and c-Myc, as well as reduced apoptosis inhibitors such as Bcl -2, in the expression of T lymphocytes in pSS patients. Other authors also described a correlation between the apoptosis receptor CD95 expression on $CD4^+$ lymphocytes and the intensity of lymphocyte apoptosis. In order to determine which T -cell populations undergo quantitative changes, $CD4^+$, $CD8^+$, and

NKT -cell populations were investigated. Supposedly, that these populations are actively involved in the pSS pathogenesis.

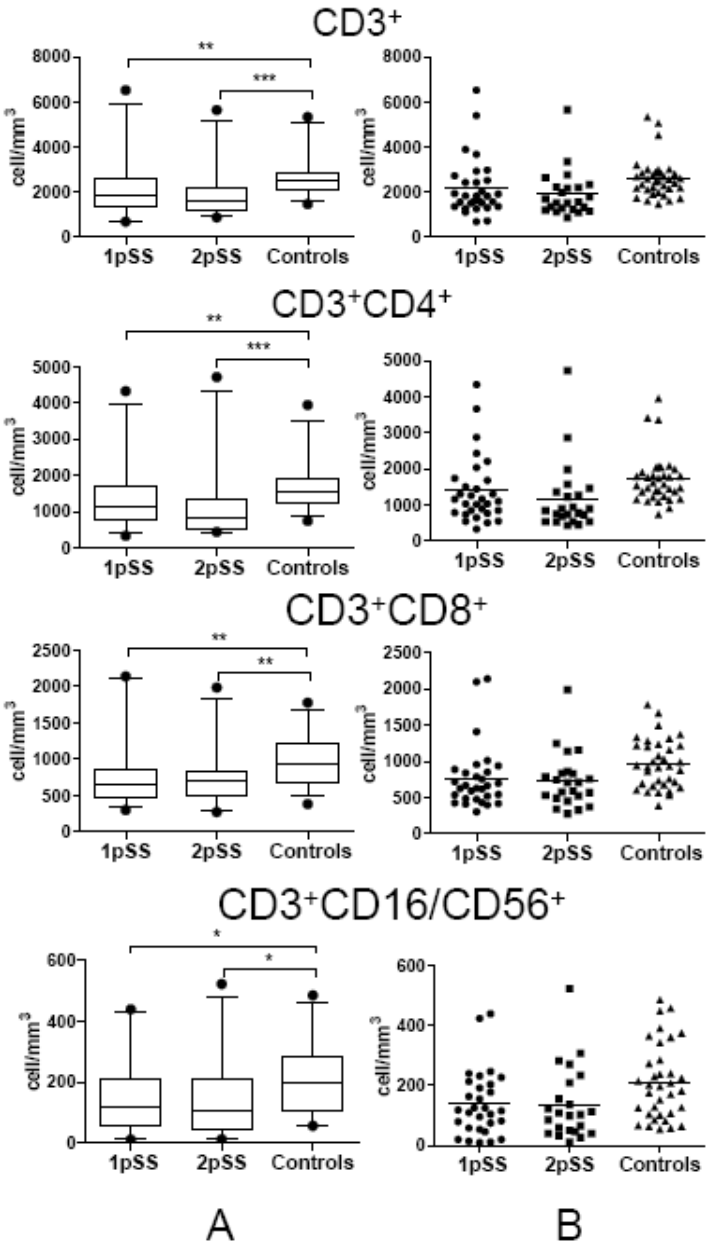

Fig. 1. *Distribution of* T c*ell populations in peripheral blood of pSS and control group patients. A, Box and whiskers indicating absolute count of cell populations, data shown as median and 5-95 percentiles; B Plots indicating the distribution of an absolute count of cell populations, each data point represents one individual patient, horizontal lines represent the mean values. * - Mann-Whitney U test*

T lymphocyte identification by CD4 and CD8 markers, showed a statistically significant decrease in the absolute count of $CD4^+$ T lymphocytes ($\mathbf{p}_{1pSS\text{-}K}$=0.0066, $\mathbf{p}_{2pSS\text{-}K}$= 0.0001) and $CD8^+$ T lymphocytes ($\mathbf{p}_{1pSS\text{-}K}$=0.0029, $\mathbf{p}_{2pSS\text{-}K}$=0.0044) in the peripheral blood of pSS patients in comparison to the control group. No significant differences were found between the 1pSS and 2pSS groups (Fig. 1.). This shows that the decline of $CD3^+$ T lymphocyte population in the peripheral blood of pSS patients is influenced by a decrease of both $CD4^+$ T and $CD8^+$ T lymphocyte absolute counts. The decrease of the total amount of $CD4^+$ T lymphocytes in the peripheral blood of pSS patients is also confirmed by other authors. Zeher et al found a significant decrease of $CD4^+$ lymphocyte counts only in the peripheral blood of patients with extra-glandular manifestations. We found a significant decrease of $CD4^+$ lymphocytes in the both pSS patient groups. Also, these authors did not found a decline of $CD8^+$ lymphocytes in the peripheral blood of pSS patients. In our study, the established $CD8^+$ lymphocyte count decrease in the peripheral blood of both pSS patient groups confirmed that $CD8^+$ lymphocytes are involved in Sjögren's syndrome pathogenesis. NKT lymphocytes also belong to $CD3^+$ T lymphocyte population, they might function as regulatory T cells, and are one of the autoimmune process preventing chains. According to literature, in patients with autoimmune rheumatic diseases the decreased NKT lymphocyte counts and functional characteristics are associated with the progression of autoimmune process and autoantibody production. The investigation of this population showed a significant decrease of NKT-cell absolute counts in the peripheral blood of pSS patients ($\mathbf{p}_{1pSS\text{-}K}$=0.0175, $\mathbf{p}_{2pSS\text{-}K}$=0.0105) in comparison to the results in the control group. The results of 1pSS and 2pSS groups were very similar (Fig. 1.). The results, obtained in this study, as well as the data presented by Kojo et al of the reduced counts of NKT lymphocytes in the peripheral blood of pSS patients support the assumption that NKT lymphocytes are implicated in the pathogenesis of pSS. Kojo et al. also found a decrease of NKT lymphocyte count in the peripheral blood of patients with other autoimmune diseases- rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis. Contrary to our results, Szodoray with colleagues detected an increased percentage of blood NKT population in the total number of lymphocytes in the peripheral blood of pSS patiens. Thus, they divided pSS

patients into groups according to the disease manifestations, and found a significantly increased percentage of peripheral blood NKT lymphocytes only in patients with extra-glandular symptoms in comparison to the pSS patients without extra-glandular manifestations and the healthy controls. No significant differences were found between the pSS patients without extra-glandular manifestations and the healthy controls.

One of the $CD4^+$ T-cell populations is Th17 lymphocytes, secreting an abundant amount of inflammatory cytokine IL-17 (also known as IL-17A). This cytokine is involved in the pathogenesis of many autoimmune diseases. It is believed that IL-17 is also involved in the pathogenesis of primary Sjögren's syndrome, as the increased IL-17 levels were found in body fluids and serum in cases of pSS. Sakai and colleagues found that minor salivary gland infiltrates in pSS patients express the highest count of Th17 lymphocytes in comparison to other Th-cell subpopulations. This study evaluated the possible changes of Th17 lymphocyte subpopulation in the peripheral blood of pSS patients.

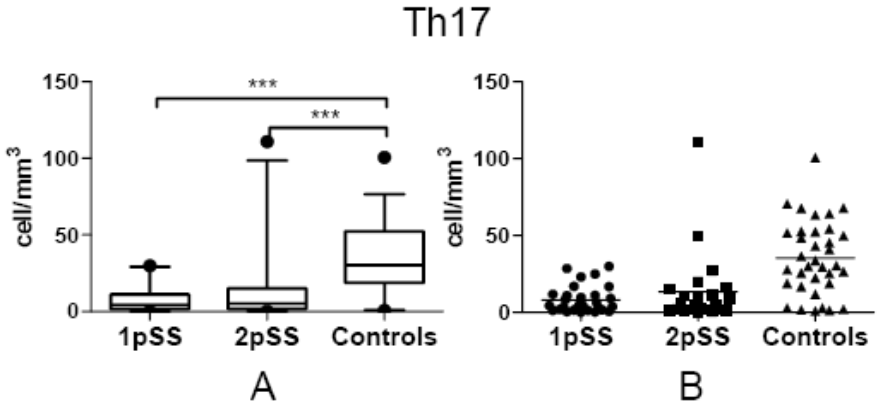

Fig. 2. *The distribution of* Th17 c*ell population in the peripheral blood of pSS and control group patients. A Box and whiskers indicating absolute count of cell population, data shown as median and 5-95 percentiles; B Plots indicating the distribution of an absolute count of cell population, each data point represents one individual patient, horizontal lines represent mean values.* * *- Mann-Whitney U test*

A statistically significant decline of Th17 lymphocyte absolute counts ($\mathbf{p}_{1pSS\text{-}K}<0.0001$, $\mathbf{p}_{2pSS\text{-}K}<0.0001$) was observed in the peripheral blood of pSS patients in comparison with the control group results. No significant difference was observed between

the 1pSS and 2pSS patient groups (Fig. 2.). IL-17 is produced not only by Th17 cells but also by NKT and NK cells, CD8 T cells, γδ T cells and a number of other blood cells. This fact can explain differences between our results and the data of other researchers. Discrepant results were also obtained by Kang et al. who determined the increase in the percentage of the Th17 lymphocytes in the peripheral blood of pSS patients. No significant differences were found in Th17 percentages between the pSS patients and the controls analyzing Th populations (see section below *Qualitatitve analysis of T helper cell populations*). Therefore, it could be assumed that the decline in Th17 lymphocyte population is associated with the total $CD3^+$ and $CD4^+$ T lymphocyte absolute count decrease in the peripheral blood of pSS patients. These observations need to be proven by a study with a bigger number of patients.

A lot of discussions among the researchers aroused evaluating the role of different T lymphocyte populations in the pathogenesis of pSS. One of the main discussion axes is regulatory T lymphocytes (Treg) identified by the CD4 marker, $CD25^i$ (i – intensive) and expression of intracellular Foxp3 marker. These lymphocytes are one of the links in the prevention of autoimmune process, as they can start the suppression mechanism to down regulate the autoimmune T lymphocyte response.

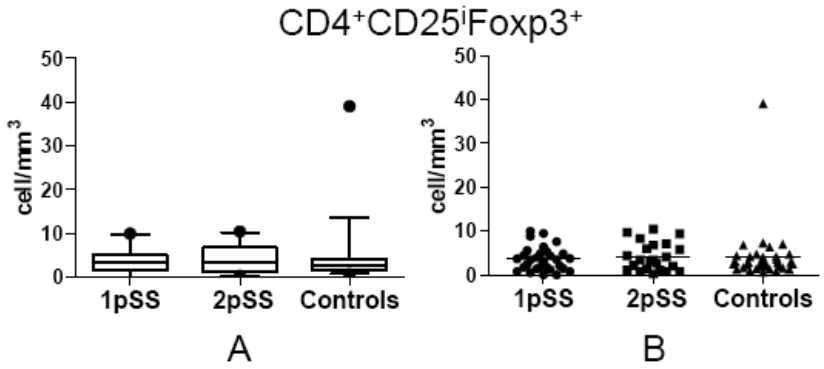

Fig. 3. *The distribution of* T_{reg} *cell population in the peripheral blood of pSS and control group patients. A, Box and whiskers indicating absolute count of cell population, data shown as median and 5-95 percentiles; B Plots indicating the distribution of the absolute count of cell population, each data point represents one individual patient, horizontal lines represent mean values.*

The results didn't show any significant differences in the absolute count of Treg lymphocytes between the groups of pSS ($p_{1pSS\text{-}2pSS}$=0.7947), also between pSS groups and a control group ($p_{1pSS\text{-}K}$=0.7273, $p_{2pSS\text{-}K}$=0.6335) (Fig. 3). Data on Treg studies found in the literature are very controversial. Scientists Liu and Li with their research teams found a declined population of Treg lymphocytes in the peripheral blood of pSS patients, when compared with the healthy controls. However, they identified Treg lymphocytes only with CD4 and $CD25^{i}$ (^{i} - an intense expression of the marker) markers. In this study we have used an intracellular marker Foxp3, that made it more specific for identification of Treg lymphocytes, as for the development of Treg FOXP3 gene expression is necessary. Sarigul and his colleagues got the same results like ours and also did not found differences when compared $CD4^{+}CD25^{+}Foxp3^{+}$ lymphocyte counts in the peripheral blood of the pSS and the healthy control group patients. Group result similarity can be explained by different inflammation degree in the salivary glands of various pSS patients that may affect the result scattering in the groups. These assumptions are partially confirmed by Christodoulou et al. After a thorough investigation of minor salivary gland biopsies and peripheral blood of pSS patients, they found a strong negative correlation between the Treg count in minor salivary gland infiltrate and peripheral blood. According to Christodoulou et al, different investigators might have received contradictory results by the wrong patient division into pSS groups regardless of the inflammation degree in the target organs.

The data of various researchers are indicating that $CD8^{+}$ lymphocytes also play an important role in the pathogenesis of Sjögren's syndrome. Some of these lymphocytes with the expression of CD57 and/or CD27 markers are known as the memory effector lymphocytes; they have large amount of A, B perforins and granzymes. These lymphocyte products are known to participate in the process of the salivary gland destruction. $CD8^{+}$ memory lymphocytes with aging (reaching a later stage of differentiation) increase the expression of CD57 marker and produce larger quantities of perforin A, B and granzymes than younger cells bearing a CD27 marker, and having a higher proliferative potential.

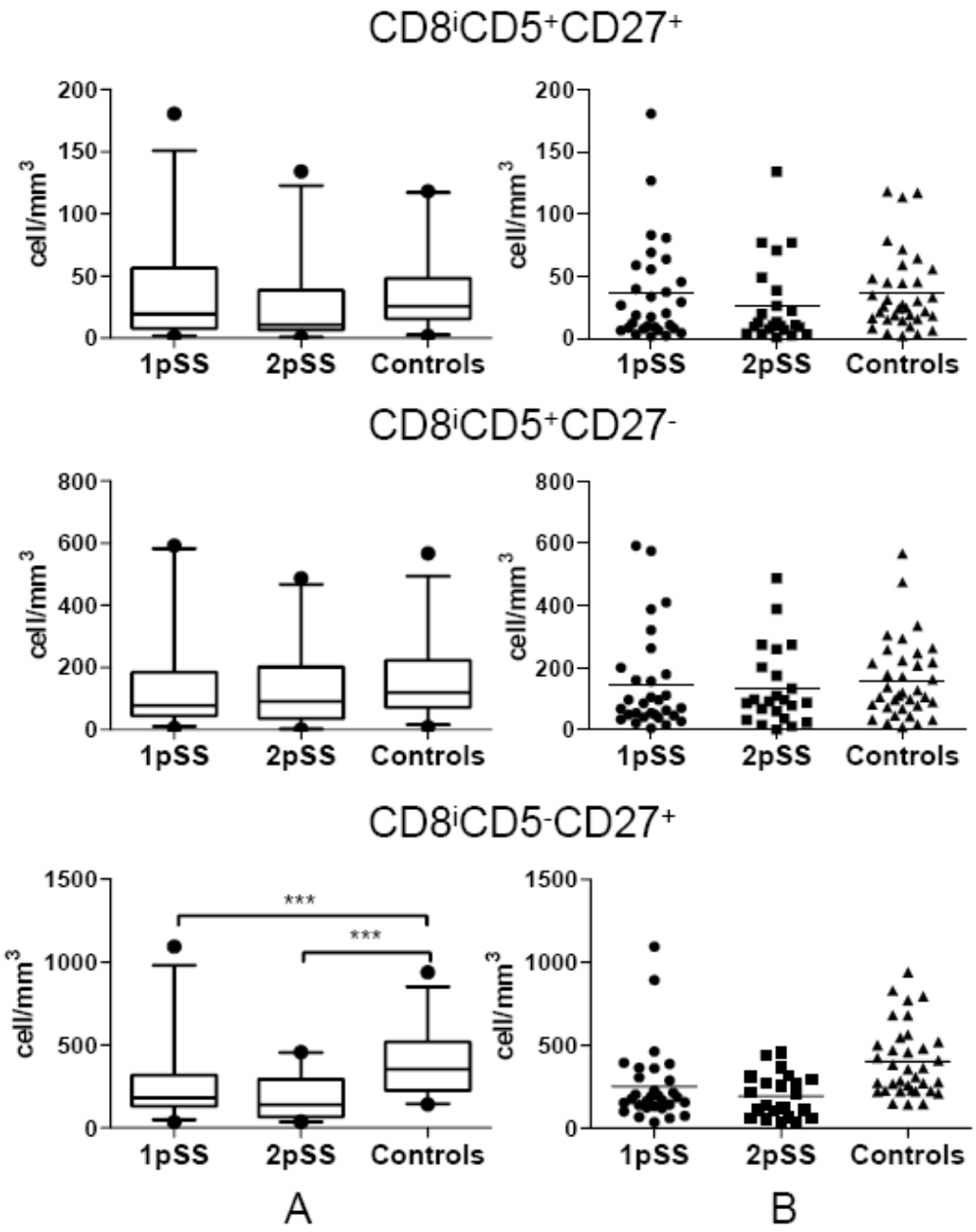

Fig. 4. *The distribution of* CD8^{i} c*ell subpopulations' in the peripheral blood of pSS and control group patients. A Box and whiskers indicating absolute count of cell populations, data shown as median and 5-95 percentiles; B Plots indicating the distribution of the absolute count of cell populations, each data point represents one individual patient, horizontal lines represent mean values. * - Mann-Whitney U test*

In this study, the quantitative assessments of CD8i memory-cell populations with CD57 and/or CD27 markers in the peripheral blood of pSS patients and controls were performed. The absolute count of $CD8^{i}CD57^{+}$ $CD27^{-}$ lymphocytes was similar in all groups ($\mathbf{p}_{1pSS\text{-}K}$=0.2137, $\mathbf{p}_{2pSS\text{-}K}$=0.2798, $\mathbf{p}_{1pSS\text{-}2pSS}$=0.9071) (Fig. 4.). It is known that in patients, suffering from autoimmune disorders, lymphocytes undergo more intense antigenic stimulation, therefore it could be expected that the number of these lymphocytes will be increased, because chronic antigenic stimulation is associated with an increase of CD57 marker on the $CD8^{i}$ population. Analyzing $CD8^{i}$ lymphocytes of the middle stage of differentiation, that are bearing both CD57 and CD27 surface markers, a decrease of $CD8^{i}CD57^{+}CD27^{+}$ lymphocyte count in 2pSS group ($\mathbf{p}_{2pSS\text{-}K}$=0.0506) was observed. No differences were found while comparing the 1pSS results with the ones of the control and 2pSS group ($\mathbf{p}_{1pSS\text{-}K}$=0.426, $\mathbf{p}_{1pSS\text{-}2pSS}$=0.404) (Fig. 4.).The analysis of $CD8^{i}$ lymphocyte populations showed a statistically significant decrease in the absolute count only of $CD8^{i}CD57^{-}CD27^{+}$ lymphocytes in the peripheral blood of pSS patients when compared with the controls ($\mathbf{p}_{1pSS\text{-}K}$=0.0002, $\mathbf{p}_{2pSS\text{-}K}$<0.0001). These cells belong to the earlier stage of $CD8^{i}$ T lymphocyte differentiation. Analyzing this lymphocyte population, no differences were found between the 1pSS and 2pSS groups ($\mathbf{p}_{1pSS\text{-}2pSS}$=0.3461) (Fig. 4.). It is likely that the reduction of $CD3^{+}$ and $CD8^{+}$ T lymphocytes in the peripheral blood of pSS patients is influenced by the decrease of this particular population of lymphocytes. The results of the study can not be compared with the ones obtained by other scientist because such studies were not found in literature. The redistribution of $CD8^{i}$ lymphocyte population shows that $CD8^{i}$ lymphocytes are actively involved in the mechanisms of pSS pathogenesis.

B lymphocytes – is one of the main lymphocyte populations actively involved in the pathogenesis of pSS. Impairment of B lymphocyte homeostasis in the pathogenesis of pSS is B cell hyperactivity manifested by auto-antibody production, hypergammaglobulinemia and formation of ectopic germinal lymphoid structures at the sites of inflammation. These manifestations are increasing the risk of B-cell lymphoma development. In order to determine possible quantitative changes of the B-cell population in the peripheral blood of pSS patients, the lymphocytes with the CD19 marker were analyzed.

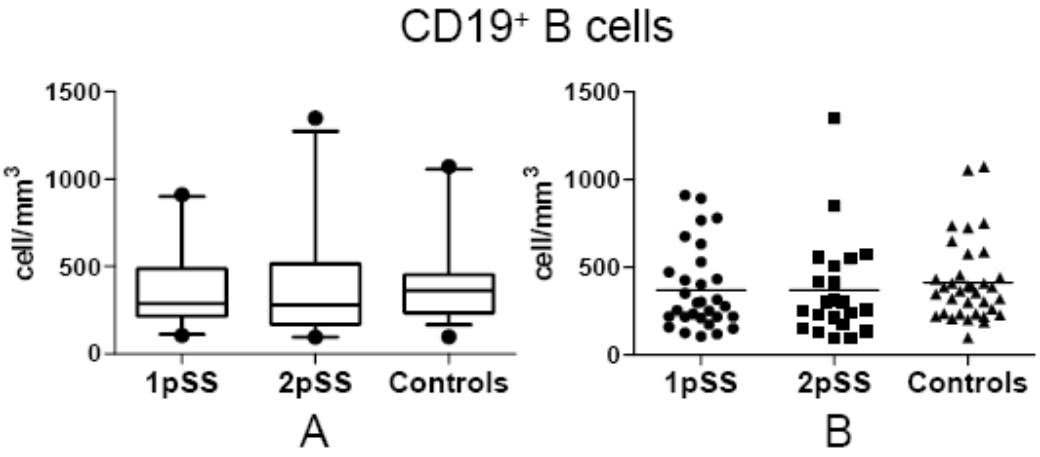

Fig. 5. *B cell distribution in the peripheral blood of pSS and control group patients. A, Box and whiskers indicating the absolute count of cell population, data shown as median and 5-95 percentiles; B Plot indicating the distribution of the absolute count of cell population, each data point represents one individual patient, horizontal lines represent mean values.*

No statistically significant quantitative differences were found between pSS patients and control patients ($\mathbf{p}_{1pSS\text{-}K}$=0.2496, $\mathbf{p}_{2pSS\text{-}K}$=0.2099, $\mathbf{p}_{1pSS\text{-}2pSS}$=0.8169) (Fig. 5.). It was difficult to compare the results with the data of other authors, because nowadays other researchers chose not to study the total population of B lymphocytes, but only the qualitative changes of B lymphocyte populations by their level of differentiation and function. Earlier studies didn't use such sensitive methods as the flow cytometry.

NK cells with their cytotoxic and regulatory properties could be considered to be one of the autoimmune processes preventing chains; supposedly, they are playing an important role in the pathogenesis of pSS. The data of other investigators show that NK cell counts are decreased in the peripheral blood of patients with pSS and systemic lupus erythematosus, in comparison to healthy control patients. A decline in their functional activity was also observed .However, some investigators got different results. Szodoray with colleagues, after study of cells with regulatory function in the peripheral blood of pSS patients, found the increased percentage of NK cells in the peripheral blood of pSS patients in comparison to healthy controls. In their study, the results of pSS patients without extra-glandular manifestations did not differ in patients with extra-glandular manifestations.

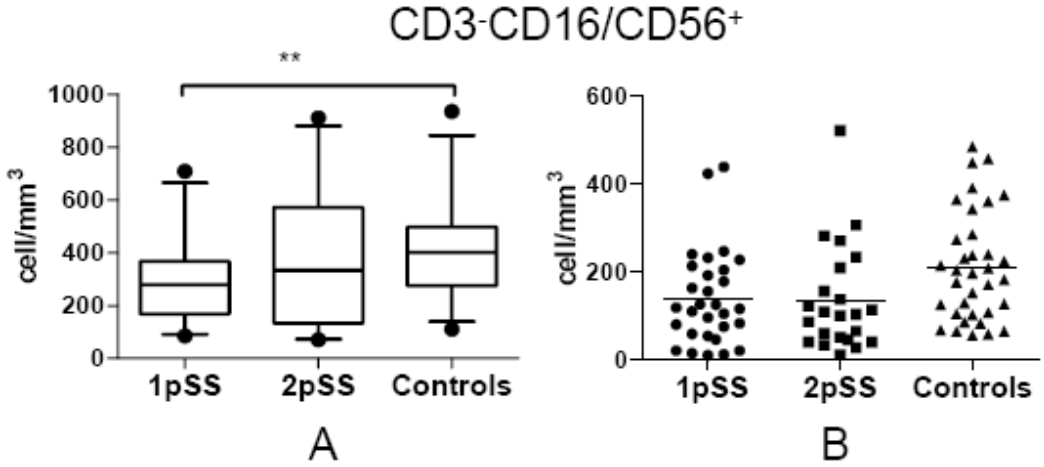

Fig. 6. *The distribution of* NK *cells in the peripheral blood of pSS and control group patients. A, Box and whiskers indicating the absolute count of cell population, data shown as median and 5-95 percentiles; B Plot indicating the distribution of an absolute count of cell population, each data point represents one individual patient, horizontal lines represent mean values. * - Mann-Whitney U test*

The results of our study showed a statistically significant absolute count reduction of NK cells ($\mathbf{p}_{1pSS\text{-}K}$=0.003) in the peripheral blood of 1pSS patient when compared with the control group. No significant differences were found in the results of 2pSS and the control group ($\mathbf{p}_{2pSS\text{-}K}$=0.266). Also, no significant differences were detected between the results of 1pSS and 2pSS groups ($\mathbf{p}_{1pSS\text{-}2pSS}$=0.404) (Fig. 6.). Different results were received by Szodoray et al.; they found the increased percentage of NK cells in the peripheral blood of pSS patients in comparison to healthy controls. A rather wide scattering of the results we found in the pSS group. A significant variance was observed in the 2pSS patient group. Probably, the 2pSS group could be divided into two separate groups of patients with increased or decreased absolute count of NK cells. We suppose that in such researches larger groups of patients should be used.

Qualitative analysis of T helper cell populations. PBMC were stained for CD4, IFN-γ, IL-4, IL-17 and T helper subsets to be determined by the flow cytometry. Upon stimulation, a similar percentage of $CD4^+$ T-cells from pSS patients expressed IFN-γ in comparison with the patients from the control group (p = 0.3389). The percentage of IL-4^+ cells within $CD4^+$

T cells from pSS was lower in comparison to that of the control group, but not significantly ($p = 0.0683$). The percentage of IL-17^+ cells within $CD4^+$ T-cells from pSS was similar in comparison to the control group ($p = 1.000$). Th17/Th1-like cells defined, as $CD4^+$ IL-$17^+IFN\gamma^+$. A clear difference was found in the increased frequency ($p = 0.0029$) of both IFN-γ and IL-17 producing Th17/Th1-like cells in pSS patients in comparison with the control group (Fig. 7A, B, C).

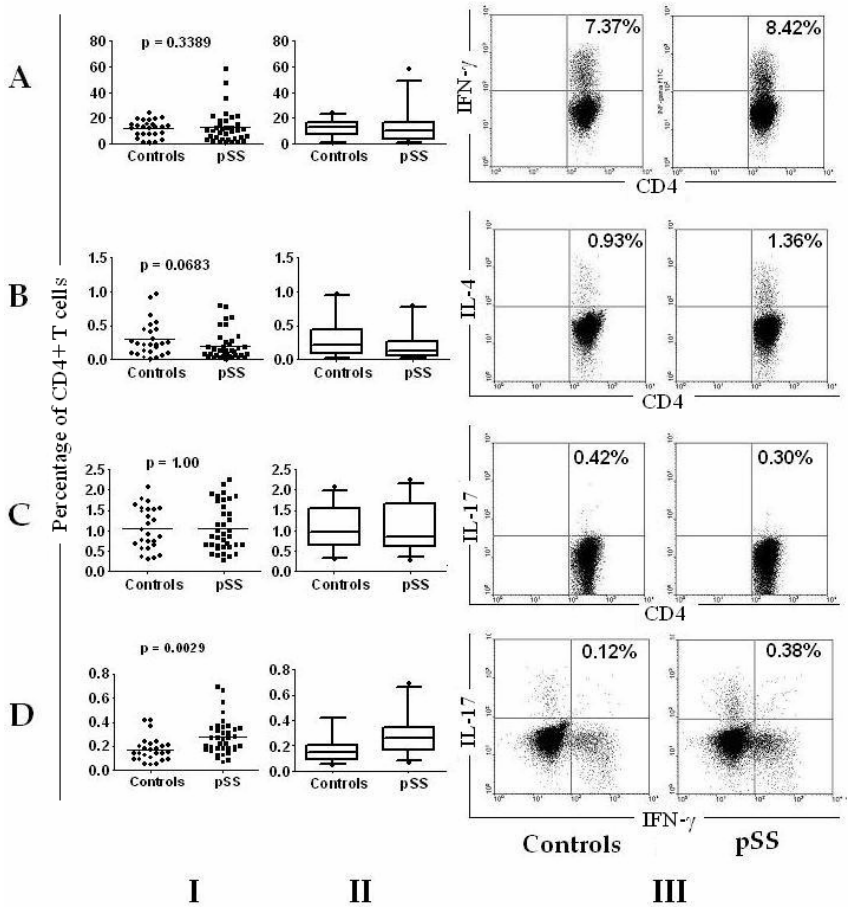

Fig. 7. *The distribution of T helper subsets A, Th1; B, Th2; C, Th17; D, Th17/Th1-like. I, Plots indicating the distribution of four T helper subsets, each data point represents one individual patient, horizontal lines represent mean values; II, Box and whiskers indicating the distribution of four T helper subsets, data shown as median and 5-95 percentiles; III, Representative dot plots of IFN-γ, IL-4, IL-17 and IL-17/IFN-γ expressing CD4+ T-cells upon stimulation.*

Correlation between the T helper subset distribution and clinical parameters among patient groups. Significant correlations among all T helper subsets were found in the control group. It was revealed that Th1 cells correlated with Th17 (r = 0.6811, p = 0.0001), Th2 (r = 0.4997, p = 0.0093), Th17/Th1-like (r = 0.6184, p = 0.0008). Th17 cells correlated with Th1, Th2 (r = 0.3981, p = 0.0440), Th17/Th1-like (r = 0.7667, p <0.0001). Th2 cells correlated with Th1, Th17, Th17/Th1-like (r = 0.4925, p = 0.0106). Th17/Th1-like cells correlated with Th1, Th17, and Th2 cells (Fig. 8A). Significantly different results were found in the pSS group. Th1 cells correlated with Th2 (r = 0.4657, p = 0.0048), Th17 (r = 0.3654, p = 0.0261) and did not correlate with Th17/Th1-like cells. Th2 cells correlated only with Th1. Th17 cells correlated with Th1 and Th17/Th1-like (r = 0.7485, p <0.0001). Th17/Th1-like cells correlated only with Th17 (Fig. 8B).

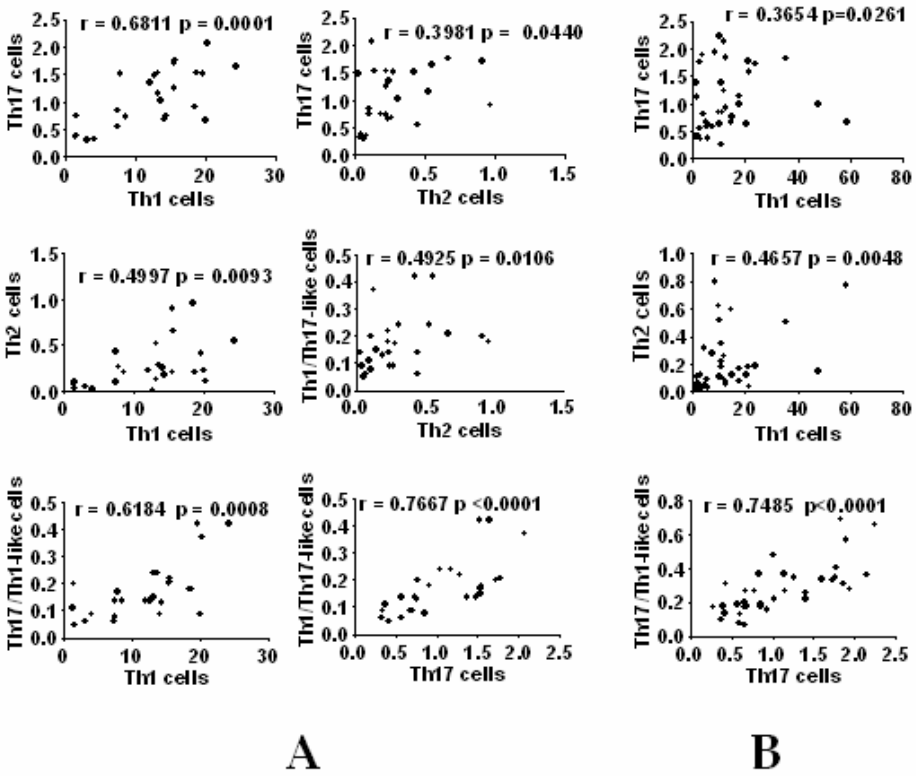

Fig. 8. *Spearman's rank correlation between T helper subset activity in PBMC, each data point represents one individual patient. A, Correlation between T helper subsets in the control group. B, Correlation between T helper subsets in the pSS group.*

We found an increased percentage of Th17/Th1-like lymphocyte ($p = 0.0029$) in the peripheral blood of patients with primary Sjögren's syndrome when compared with the control group (Fig. 7 D). Th17/Th1-like lymphocytes are recently discovered new Th subpopulation. Müller et al. also found $CD4^+$ IL-17^+/IFN-γ^+ lymphocytes in the peripheral blood of patients with Crohn's disease and inflammatory bowel tissue. Rovedatti and others detected an increased percentage of these cells in patients suffering from chronic inflammatory bowel disease in biopsy of intestinal material in comparison to the results in the control group. Kebir et al. presented data which indicated an increase in $CD4^+$IL-17^+/IFN-γ^+ lymphocyte percentage in the central nervous system of experimental allergic encephalomyelitis in the mouse model and found that these cells can more easily pass through the brain barrier and are involved in the active inflammatory processes. Pathogenic role of lymphocytes, shown in other chronic inflammatory disease processes, allows us to assume that an increased Th17/Th1-like rate in the peripheral blood of pSS patients indicates that these cells are involved in the pathogenic mechanisms of pSS. After analyzing the relationship among the Th populations in the controls, it can be said that the distribution of four populations in the peripheral blood is closely related and generally increases a more active immune process in the body. In the peripheral blood of pSS patients, the picture is quite different. Th1 cells correlated with Th2, Th17 and did not correlate with Th17/Th1-like cells. Th2 cells correlated only with Th1. Th17 cells correlated with Th1 and Th17/Th1-like. Th17/Th1-like cells correlated only with Th17. Such differences, observed between the groups, clearly indicate an impaired T-cell homeostasis in the pathogenesis of the primary Sjögren's syndrome. This imbalance could be caused by the decreased percentage ofTh2 and significantly increased Th17/Th1-like lymphocyte percentage.

Investigation of IgG, κ and λ FLC, BAFF and complement C4 fragment C4d serum levels. Humoral biomarkers play an important role in the diagnosis of systemic autoimmune diseases, evaluation of the disease activity in these phases, also in the treatment strategies and further development of the disease prognosis. Clinical significance of these biological markers depends on how accurately they reflect the processes going on in the body. It is important to assess how they are influenced by the individual characteristics of the patient

therapeutic intervention or any other pathological processes. Several such biomarkers (BAFF, κ and λ FLC, C4d and IgG), were analyzed; their association with the distribution of lymphocyte populations (absolute counts) in patients with primary Sjögren's syndrome (pSS) without (1pSS) or with (2pSS) extra-glandular manifestations was tested. Our findings may indicate some differences in the ongoing mechanism of pSS patients without (1pSS) or with (2pSS) extra-glandular manifestations. Serum BAFF levels were significantly higher in 2pSS group ($p = 0.004$) as compared with the control group. The increase of BAFF level in the 1pSS group was not statistically significant ($p = 0.09$). No significant differences in serum BAFF level were observed between the 1pSS and 2pSS groups ($p = 0.2833$). A significant increase of κ FLC level was observed only in the 2pSS group ($p = 0.001$) as compared with the control group. Moreover, the level of κ FLC was significantly higher in the 2pSS group ($p = 0.0115$) as compared with the 1pSS group. The level of λ FLC was significantly higher only in the 2pSS group ($p = 0.012$) in comparison to the control group. FLC levels were higher in the pSS patients with extra-glandular involvement than in those without. The serum level of C4d was comparable in all three groups: 1pSS vs. controls ($p=0.189$), 2pSS vs. controls ($p= 0.757$) and 1pSS vs. 2pSS ($p = 0.356$).The concentration of IgG was significantly elevated in the serum of pSS patients in comparison to the control group, in 1pSS ($p = 0.001$) and 2pSS ($p = 0.0003$). No significant difference in IgG level was observed between the 1pSS and 2pSS groups ($p = 0.724$). All data are shown in Table 1.

Table 1. Serum factor levels in pSS patients and controls.

	1 pSS group (n=35)		**2 pSS group (n=23)**		**Controls (n=22)**		**p* I-II**	**p I-III**	**p II-III**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**			
BAFF(ng/ml)	4.273	1.768	4.941	2.465	3.290	1.105	0.283	**0.004**	0.09
κ (mg/l)	9.945	5.476	15.569	9.459	6.949	2.551	**0.012**	0.059	**0.001**
λ (mg/l)	10.297	9.298	16.259	14.224	6.937	3.206	0.108	0.119	**0.012**
κ/λ	1.16	0.56	1.35	0.75	1.06	0.34	0.150	0.928	0.256
C4d (μg/ml)	2.22	1.55	2.46	1.59	2.86	1.90	0.757	0.189	0.356
IgG (g/l)	122.40	74.64	129.96	59.63	60.69	63.51	0.724	**0.001**	**0.0003**

*- Mann-Whitney U test

Correlations between humoral factors. Significant correlations were observed in the 1pSS group: BAFF correlated with κ ($p=0.002$; $r=+0.513$) and λ FLC ($p=0.004$; $r=+0.479$), κ correlated with λ ($p<0.001$; $r=+0.815$) and IgG ($p=0.022$; $r=+0.423$). Also a strong correlation was observed between the autoantibodies anti-Ro/SSA and anti-La/SSB ($p<0.001$; $r=+0.700$). In the 2pSS group we found correlations between BAFF and anti-La/SSB ($p=0.027$; $r=+0.494$), λ correlated with κ ($p=0.001$; $r=+0.630$), IgG correlated with anti-Ro/SSA ($p=0.029$; $r=+0.476$) and anti-Ro/SSA with anti-La/SSB ($p=0.021$; $r=+0.511$). Ratio of κ/λ negatively correlated with λ ($p=0.002$; $r=-0.612$) and C4d ($p=0.006$; $r=-0.553$) levels. Elevated levels of C4d tended to associate negatively with the concentration of autoantibodies anti-Ro/SSA ($p=0.051$; $r=-0.431$) and anti-La/SSB ($p=0.054$; $r=-0.436$); however, these associations were not statistically significant.

Correlation between the distribution of PBMCs and humoral factors. Only in the 1pSS group the correlation was observed between the BAFF and PBMC distribution. BAFF level negatively correlated with the absolute counts of CD3+ ($p=0.045$; $r=-0.369$), CD3+CD4+ ($p=0.038$; $r=-0.380$), CD3+CD8+ ($p=0.014$; $r=-0.445$) and B cells ($p=0.026$; $r=-0.406$). κ/λ ratio correlated positively with the absolute counts of NK ($p=0.037$; $r=+0.383$) and B cells ($p=0.024$; $r=+0.412$). The level of C4d correlated with the absolute counts of CD3+CD8+ ($p=0.023$; $r=+0.414$) and NKT cells ($p=0.002$; $r=+0.550$). Negative correlation was observed between the IgG level and absolute count of Th17 cells. In 2pSS group, BAFF and C4d levels did not correlate with the PBMC distribution. The level of λ FLC negatively correlated with the absolute counts of CD3+CD4+ ($p=0.049$; $r=-0.424$) and CD3+CD4+/CD3+CD8+ cells ratio ($p=0.047$; $r=-0.427$). The IgG level negatively correlated with the absolute counts of CD3+ ($p=0.0032$; $r=-0.627$), CD3+CD4+ ($p=0.016$; $r=-0.531$) and CD3+CD4+/CD3+CD8+ cell ratio ($p=0.044$; $r=-0.454$).

One of the potential biomarkers in the pathogenesis of Sjögren's syndrome is the B-cell activating factor (BAFF) a member of the tumor necrosis factor (TNF) superfamily. It is a potent survival factor for B cells, and it plays an essential role in the peripheral B-cell homeostasis. BAFF induces cell proliferation and differentiation. However, it can also

induce the loss of tolerance and may be associated with lymphoma development. An increased BAFF level may not always reflect the disease activity. An increased level of BAFF in the blood serum of pSS patients is found after Rituximab therapy that eliminates B lymphocytes. This occurs because of actively produced BAFF by monocytes. Monocytes, after the reduction of B lymphocytes, do not get the negative regulatory signal from B lymphocytes and produce larger amounts of BAFF, thus becoming the perpetrators of the increased BAFF level in serum. Therefore, analysis of the BAFF level in serum must be done with a responsibility, taking into account the methodologies used in the treatment of patients. Although Zhu et al. assume that BAFF protects not only B but also T lymphocytes from apoptosis; our data show a negative correlation between the BAFF expression and the absolute number of T lymphocytes in the peripheral blood of pSS patients. Further studies must be performed to reveal the relationships between BAFF and T-lymphocytes in the pathogenesis of pSS.

Recent researches have shown that free immunoglobulin light chains (FLC) κ and λ are involved in the immunological mechanisms of regulation; they act as signaling or anti-inflammatory molecules. Quantitative study of these molecules and their ratio (κ/λ) is a sensitive and specific method for the detection of FLC diseases. In 1998, was revealed that free immunoglobulin light chains in serum can be predictive markers of lymphoma development in the pathogenesis of pSS. In those studies, the pSS patients were not divided into groups with and without extra-glandular manifestation. The patients with extra-glandular manifestations had higher levels of free κ and λ light chains, compared with the ones with only glandular manifestations. Revealed associations between FLC and the components of cellular immunity allow the obtained changes of FLC concentration to use as a biomarker for evaluation of the course of the disease in individual pSS patients.

C4d is a fragment of the human complement C4, characterized by its stability and easy identification. The complement system dysfunction, low amounts of C3 and C4, are identified as an unfavorable course of the disease like lymphoma and/or premature death. C4d is an indirect footprint or marker of antibody response, which indicates recent complement activation through the classical pathway. At the moment C4d is already a good

marker used in transplantation, where the accumulation of C4d along the peritubular capillaries is associated with a poor graft survival. Also, it is described as a possible criterion in the diagnosis of systemic lupus erythematosus. According to the results of our study, this fragment may be more informative in the disease course assessment of an individual patient, especially when it is compared with other clinical features. This is confirmed by a more detailed study of one patient from the 1pSS group, when blood samples, taken throughout the year, demonstrated a decrease of the C4d fragment concentration (from 2.07 mg / ml (2011.04.13), up to 1.33 mg / ml (2011.09.13) and 0.73 mg / ml (2011.11.21)). Also, a serum sample from one 2pSS group patient showed 0 value of C4d concentration. This, according to the literature, shows complete complement exhaustion and an increased risk of death. Polyclonal B cell activation is one of the most typical immunoregulational disorders, which is also present in the pathogenesis of pSS. This disorder is accompanied by hypergammaglobulinemia and the production of various auto-antibodies. These immune dysregulations are associated with an increased risk of developing lymphoma among the pSS patients. Study results showed that serum level of IgG was significantly increased in the1pSS and 2pSS patient groups when compared with the control group results. A statistically significant difference between the pSS patients with extra-glandular manifestations and without wasn't observed in the analysis of serum IgG concentration. Different relationships between hypergammaglobulinemia and the changes in the distribution of lymphocyte populations possibly show differences in the mechanisms of pathogenesis in the 1pSS and 2pSS groups. All these findings suggest that in the presence of extra-glandular manifestations the pSS patients undergo more intense stimulation of B cells and stronger dysregulations in the immune system.

Investigation of IL-27 and IL-35 levels in serum of pSS patients. In recent years, there is a big interest in investigation of IL-27 and IL-35 regulatory impact for autoimmune processes in the autoimmune disease models. Therefore, the investigation of these interleukins in the sera of pSS patients was one of the immune system research plans.

Study results showed a statistically significant increase of IL-27 ($p < 0.001$) and IL-35 ($p < 0.001$) in serum of pSS patients in comparison to the control group results (Table 2).

Table 2. Interleukin levels in the serum of pSS patients and controls

	Controls (n = 50)			*pSS (n =50)*			p*
	Mean	Median	SD	Mean	Median	SD	
IL-27 (pg/ml)	2255	2112	1824	62558	20085	80418	**<0.001**
IL-35 (pg/ml)	3497	3231	2067	126059	42556	184382	**<0.001**

*- Mann-Whitney U test

Similar results were found by Yoshizaki et al. investigating IL- 27 expression in patients with systemic sclerosis therapy. Elevated IL- 27 levels were found in the serum of patients with systemic sclerosis compared with healthy controls. Also, the correlation between IL- 27 elevations and the increased B cell synthesized IgG content, as well as the increase in IL -17 content, synthesized by CD4 lymphocytes were detected. IL- 27 levels were higher in the blood of those patients who had a longer duration of illness. Many studies of other investigators, examining IL- 27 functions in the regulation of the immune response are controversial. Some authors describe IL- 27 as a protective, and some as an inflammatory humoral factor. Lee and colleagues studying the Sjögren's syndrome in a mouse model found that in mice received intravenous injections of viral vectors those expressed IL- 27, in serum occurred a long-term increase of IL- 27 level, what resulted a milder course of the disease. Also reduced IL -17 serum levels and ANA titers as well as alleviated clinical symptoms are associated with the impaired secretion of saliva. Despite the increased serum levels of IL- 27 in patients with pSS, the present scientific understanding of its role in the pathogenesis of pSS is very limited. More detailed studies should be performed evaluating what role - protective or pathogenic this interleukin plays in the pathogenesis of pSS.

IL -35 functions are very similar to that of IL- 27. This interleukin may activate effector cells in the acute infection phase. Also, in the onset of an active inflammatory phase it can selectively suppress effector cells by inducing Treg lymphocytes; thus preventing the development of the autoimmune response. There are no present data about the role of IL-35

in the pathogenesis of pSS, but the increase of this interleukin, found in the serum of pSS patients suggests that it also plays a role in the pathological process of pSS.

Conclusions

1. Lymphocytopenia observed in peripheral blood of primary Sjögren's syndrome patients was due to decreased absolute counts of T and NK cells. The decline of T cell absolute count is influenced by decreased absolute counts of NKT, $CD3^+CD4^+$ and $CD3^+CD8^+$ cell populations, in which the decreased absolute counts of $CD4^+IL\text{-}17^+$ and $CD8^{i}CD57^-CD27^+$ lymphocyte subpopulations, respectively were observed. Analysis of the B-cell population in peripheral blood didn't show any significant differences between the pSS patients and control groups.

2. T-cell homeostasis in the pathogenesis of the primary Sjögren's syndrome is impaired. Th2 percent decrease tendency and significantly increased (**p**=0.0029) Th17/Th1-like lymphocyte percent causes this imbalance.

3. Primary Sjögren's syndrome patients with extra-glandular manifestations have bigger imbalance in expression of humoral factors:

   3.1. Statistically significant increased concentration of BAFF level was observed in the serum of primary Sjögren's syndrome patients with extra-glandular manifestations (**p**=0.004), but no correlation was observed between serum BAFF level and cellular immunity components. In the group of primary Sjögren's syndrome patients with only glandular manifestations BAFF level negatively correlated with the absolute counts of B and T lymphocytes ($CD4^+$, $CD8^+$ and it's subpopulation $CD8^{i}CD57^-CD27^+$).

   3.2. Increased levels of κ and λ FLC in serum of the pSS patients are showing hyperactivity of B cells and disbalance in production of their products, but only in patients with extra-glandular manifestations had significantly increased serum levels of κ and λ FLC (κ **p**=0.001, λ **p**=0.012).

3.3. Level of serum IgG was significantly increased in both patients groups - with glandular (**p**=0.001) and extra-glandular manifestations (**p**=0.0003).

4. The concentration of C4d was comparable in all groups. However, in the 2pSS group the inverse correlation between C4d and κ/λ FLC ratio was revealed, and also a tendency of negative association with concentrations of anti-Ro/SSA (p=0.051; r=-0.431) and anti-La/SSB (p=0.054; r=-0.436) auto-antibodies was detected. C4d associations with these disease activity markers are showing potential of the C4d as a marker for disease course evaluation.

5. Detected increase of interleukins IL-27 and IL-35 in serum of primary Sjögren's syndrome patients indicates that they have an important role in the pathological process of pSS.

List of publications

1. **G. Sudzius**, D. Mieliauskaite, I. Butrimiene, A. Siaurys, Z. Mackiewicz, I. Dumalakiene. Activity of T-helper cells in patients with primary Sjögren's sindrome. In Vivo. 2013. 27: 263-268. Article is featured online on Global Medical Discovery [ISSN 1929-8536] (http://globalmedicaldiscovery.com)

2. **G. Sudzius**, D. Mieliauskaite, A. Siaurys, R. Viliene, I. Butrimiene, D. Characiejus, I. Dumalakiene. Could the complement component C4 or its fragment C4d be a marker of the more severe conditions in patients with primary Sjögren's syndrome? Rheumatol Int (2013) j: 1–9. DOI 10.1007/s00296-013-2885-y

Participations at conferences

1. G. Sudzius, I. Dumalakiene, A. Siaurys, D. Mieliauskaite, I. Butrimiene, Z. Mackiewicz. Activity of T helper cells in patients with primary Sjögren's syndrome. European Congress of Immunology, ECI 2012 Glasgow, JK. Abstracts of poster session on 5-8 September, 2012. Thesis printed: Immunology, 2012, 137 (Suppl. 1), p. 345.

2. **Sudzius G**, Characiejus D, Mieliauskaite D, Siaurys A, Butrimiene I and Dumalakiene I. Distribution of peripheral blood lymphocyte populations in patients with primary Sjögren's syndrome. Conference Abstract: 15th International Congress of Immunology (ICI). Thesis printed: Frontiers in Immunology, 2013.

3. Dumalakiene I, Siaurys A, Mieliauskaite D, Butrimiene I, Characiejus D, Mauricas M and **Sudzius G**. BAFF, κ and λ free light chains, C4d and IgG in serum and relations with peripheral blood lymphocyte populations in patients with primary Sjögren's syndrome. *Conference Abstract: 15th International Congress of Immunology (ICI)*. Thesis printed: Frontiers in Immunology, 2013.

Reziumė

Sjögreno sindromas (SS) arba autoimuninis epitelitas – tai kompleksinė lėtinė autoimuninė liga, kuriai būdinga egzokrininių liaukų epitelio infiltracija autoreaktyviais limfocitais ir specifiniai anti-Ro/SSA ir anti-La/SSB autoantikūnai kraujo serume. Ligos pasireiškimo spektras platus - nuo specifinių organų (seilių, ašarų ir kitų egzokrininių liaukų) lokalaus pažeidimo iki sisteminio pasireiškimo, kai pažeidžiami kiti organai ir audiniai, ir limfomos išsivystymo. Sjögreno sindromas skirstomas į pirminį ir antrinį SS, kai kartu su kita autoimunine liga nustatomas ir SS. Paprastai pirminiu Sjögreno sindromu sergantys pacientai skirstomi į dvi grupes: pacientai kuriems pažeidžiamos tik ašarų ir seilių liaukos (liaukinė raiška (1pSS)), ir pacientai kuriems sistemiškai pažeidžiami kiti organai ir audiniai (ekstra-liaukinė raiška (2pSS)).

Pastaruoju metu ypatingai intensyviai tiriami veiksniai, kurie gali paskatinti išsivystyti sindromą. Nepaisant intensyvių imuninės sistemos tyrimų, Sjögreno sindromo patogenezės modelis nėra pilnai aiškus. Tiriama limfocitų populiacijų sudėtis seilių liaukų infiltrate, humoraliniai veiksniai ir jų ryšiai su imuninės sistemos ląstelėmis. Įvairių autorių literatūroje skelbiami rezultatai dažnai yra prieštaringi. To priežastimi gali būti ir tyrimų nenuoseklumas, skirtingi tyrimo modeliai/sistemos, taip pat negalima atmesti sergančiųjų demografinių skirtumų. Mažiau dėmesio skiriama imuninės sistemos ląstelių tyrimams periferiniame kraujyje, o tyrimų rezultatai dažnai taip pat yra prieštaringi. Pirminiu SS sergančiųjų kraujyje būdinga limfopenija. Siekiant nustatyti to priežastis atlikta nemažai tyrimų, tačiau trūksta detalesnių tyrimų, atskleidžiančių kokių ląstelių populiacijų padaugėja/sumažėja, bei kokių ląstelių subpopuliacijų sąskaita, tai vyksta. Trūksta tyrimų, kurie susietų imuninės sistemos ląstelių populiacijų pokyčius ir humoralinių veiksnių raišką. Periferinis kraujas yra patogus tyrimo objektas, todėl naujų biožymenų paieška kraujyje ir serume yra itin aktuali, ypač neseniai pradėjus taikyti biologinius preparatus autoimuninių ligų gydymui.

Šiame darbe buvo atlikta kompleksinė, detali B, NK, T limfocitų populiacijų ir humoralinių veiksnių analizė sergančių pSS pacientų periferiniame kraujyje, ko iki šiol nebuvo padaryta. Nustatytas statistiškai patikimas absoliutaus T limfocitų kiekio sumažėjimas pirminiu Sjögreno sindromu sergančiųjų pacientų be ekstra-liaukinės raiškos ir

su ekstra-liaukine raiška grupėse lyginant su kontrolinės grupės tiriamųjų rezultatais. Patikimo skirtumo tarp 1pSS ir 2pSS grupių nenustatyta. T ląstelių absoliutaus kiekio sumažėjimas pSS pacientų grupėse gali būti susijęs su ląstelių migracija į uždegiminius židinius ir/arba su intensyvesne periferinių T limfocitų apoptoze. Tokias prielaidas patvirtina ir kiti tyrėjai nustatę padidėjusį T limfocitų apoptozės intensyvumą pSS pacientų periferiniame kraujyje. Siekiant išsiaiškinti kurių T limfocitų populiacijų kiekybiniai pokyčiai nulemia pacientų, sergančių pSS, bendro $CD3^+$ T ląstelių kiekio sumažėjimą periferiniame kraujyje, toliau tyrėme $CD4^+$, $CD8^+$ ir NKT limfocitų populiacijas, kadangi manoma, kad šios populiacijos aktyviai dalyvauja pSS patogenezės mechanizmuose. Nustatytas statistiškai patikimas absoliutaus $CD4^+$ ir $CD8^+$ T limfocitų kiekio sumažėjimas pacientų, sergančių pSS, periferiniame kraujyje lyginant su kontrolinės grupės pacientų rezultatais. Patikimo skirtumo tarp 1pSS ir 2pSS grupių nenustatyta nei $CD4^+$, nei $CD8^+$ T limfocitų populiacijoje. Tai rodo, kad bendros $CD3^+$ T limfocitų populiacijos sumažėjimo tendencijai pSS sergančių pacientų periferiniame kraujyje įtakos turi tiek $CD4^+$ T, tiek $CD8^+$ T limfocitų absoliutaus skaičiaus sumažėjimas. $CD4^+$ ir $CD8^+$ T limfocitų kiekio sumažėjimas pSS sergančių pacientų periferiniame kraujyje patvirtina, kad šie limfocitai aktyviai dalyvauja Sjögreno sindromo patogeneziniame mechanizme. Vis dėlto, nustatyti ar šių ląstelių absoliutaus kiekio sumažėjimas pSS pacientų grupėse gali būti susijęs su ląstelių migracija į uždegiminius židinius ir/ar su intensyvesne periferinių T limfocitų apoptoze reikalingi papildomi tyrimai.

NKT limfocitai, taip pat priskiriami $CD3^+$ T limfocitams, yra viena iš autoimuninio proceso prevencijos grandžių. Sergant autoimuninėmis reumatinėmis ligomis NKT limfocitų skaičiaus sumažėjimas ir funkcinių savybių susilpnėjimas siejamas su autoimuninio proceso progresavimu ir auto-antikūnų gamyba. Tiriant šią populiaciją, taip pat buvo nustatytas reikšmingas NKT limfocitų kiekio sumažėjimas pSS sergančių pacientų periferiniame kraujyje lyginant su kontrolinės grupės pacientų rezultatais. NKT limfocitų absoliutus skaičius 1pSS ir 2pSS grupėse buvo labai panašus. Gauti rezultatai patvirtina NKT limfocitų dalyvauja pSS patogenezėje.

$CD4^+$ populiacija yra gana heterogeniška ir viena iš šių limfocitų populiacijų yra Th17 limfocitai, gausiai sekretuojantys uždegiminį citokiną IL-17 (dar žinomą kaip IL-17A). Šis citokinas dalyvauja daugelio autoimuninių ligų patogenezėje. Manoma, kad IL-17 dalyvauja ir pirminio Sjögreno sindromo patogenezėje, kadangi pSS atveju randami padidinti IL-17 kiekiai. Literatūroje yra duomenų, kad sergančių pSS mažųjų seilių liaukų infiltratuose Th17 limfocitų kiekis yra didžiausias lyginant su kitomis Th limfocitų populiacijomis.

Kita $CD4^+$ limfocitų populiacija tai daug diskusijų tarp tyrėjų, vertinant skirtingų T limfocitų populiacijų vaidmenį Sjögreno sindromo patogenezėje, sukėlę T reguliaciniai (Treg) limfocitai, identifikuojami pagal CD4 žymenį - CD25 ir viduląstelinį Foxp3 žymenį. Šie limfocitai yra viena iš autoimuninio proceso prevencijos grandžių, dalyvaujančių autoimuninių T limfocitų atsako slopinime.

Mūsų darbe buvo įvertinti Th17 ir Treg limfocitų populiacijų galimi pokyčiai pSS sergančių pacientų periferiniame kraujyje.

Nustatytas statistiškai patikimas absoliutaus Th17 limfocitų kiekio sumažėjimas pSS sergančiųjų pacientų periferiniame kraujyje lyginant su kontrolinės grupės pacientų rezultatais. Patikimo skirtumo tarp 1pSS ir 2pSS pacientų grupių nebuvo nustatyta. Procentinės Th17 dalies tarp Th limfocitų įvertinimas neparodė reikšmingų skirtumų tarp pSS ir kontrolinių tiriamųjų (žr. žemiau T ląstelių pagalbininkių kokybinis įvertinimas periferiniame kraujyje), todėl galima būtų daryti prielaidą, kad Th17 limfocitų populiacijos sumažėjimo tendencija susijusi su bendru $CD3^+$ ir $CD4^+$ T limfocitų absoliutaus skaičiaus sumažėjimu periferiniame kraujyje.

Gauti rezultatai tiriant Treg limfocitus neparodė statistiškai patikimo absoliutaus šių limfocitų skaičiaus skirtumo nei tarp abiejų pSS grupių, nei tarp atskirų pSS grupių lyginant jas su kontrolinės grupės pacientų rezultatais, nors galima rasti ir kitokių tyrimo rezultatų. Tokius skirtingus rezultatus galimai nulemia įtraukimas į grupes pSS pacientų su skirtingu uždegimo laipsniu seilių liaukose, kas ir sąlygoja rezultatų išsibarstymą grupėse.

Manoma, kad autoimuninių susirgimų, tame tarpe ir pirminio Sjögreno sindromo, patogenezėje yra sutrikusi Th limfocitų homeostazė. Th ląstelių kokybinio įvertinimo periferiniame kraujyje etape tyrėme keturias Th populiacijas (Th1, Th2, Th17 ir Th17/Th1-

like), siekiant išsiaiškinti ir galimai patvirtinti arba paneigti sutrikusios Th limfocitų homeostazės pSS patogenezėje prielaidą. Ištyrę Th populiacijų pasiskirstymą periferiniame kraujyje, aptikome statistiškai patikimą palyginti neseniai atrastos Th17/Th1-*like* limfocitų subpopuliacijos procento padidėjimą pirminiu Sjögreno sindromu sergančių pacientų periferiniame kraujyje lyginant su kontrolinės grupės rezultatais. Kitų tyrėjų nustatytas šių limfocitų aktyvus patogeninis vaidmuo kitų lėtinių ligų uždegiminiuose procesuose, leidžia ir mums iškelti prielaidą, kad pSS periferiniame kraujyje padidėjęs Th17/Th1-*like* limfocitų procentas parodo, kad šios ląstelės dalyvauja pSS patogeneziniuose mechanizmuose. Th2 limfocitų procentas sergančių pSS pacientų periferiniame kraujyje buvo sumažėjęs lyginant su kontroline grupe, tačiau skirtumas nebuvo statistiškai patikimas. Tiriant Th1 ir Th17 limfocitų populiacijas patikimo skirtumo nenustatyta. Analizuojant mūsų tyrimo duomenų išsibarstymą, galima įžvelgti, kad kiekvienoje grupėje pacientai pasidalina į dvi populiacijas. Galima to prielaida, vyksta pastovi limfocitų recirkuliacija į uždegimo židinius ir atgal į kraują. Esant aktyviam uždegiminiam procesui ląstelės aktyviai migruoja į uždegimines vietas. Esant ligos remisijai jos taip pat gali grįžti atgal į periferinį kraują. Stebima Th2 limfocitų procento sumažėjimo tendencija ir patikimas Th17/Th1-*like* limfocitų procento padidėjimas, aiškiai parodo, kad pirminio Sjögreno sindromo patogenezėje yra sutrikusi T ląstelių homeostazė.

Literatūroje yra duomenų, kad $CD8^+$ limfocitai taip pat aktyviai dalyvauja Sjögreno sindromo patogenezėje. Dalis šių limfocitų su jiems būdingų žymenų CD57 ir/arba CD27 ekspresija, laikomi atminties efektoriniais limfocitais, pasižyminčiais didesne perforinų A ir B, granzimų sinteze. Būtent šie limfocitų sintezės produktai ir dalyvauja seilių liaukų destrukcijos procese. $CD8^+$ atminties limfocitams senėjant (pasiekiant vėlesnę diferenciacijos stadiją), didėja CD57 žymens raiška ir jie gamina didesnius kiekius perforinų A ir B, granzimų, nei jaunesnės, CD27 žymenį turinčios ir didesniu proliferacijos potencialu pasižyminčios ląstelės. Savo tyrime atlikome $CD8^i$ (^{i}-intensyvus) atminties limfocitų populiacijų, turinčių CD57 ir/arba CD27 žymenis, kiekybinį įvertinimą tiriamųjų periferiniame kraujyje. Absoliutus $CD8^iCD57^+CD27^-$ limfocitų skaičius periferiniame kraujyje tarp grupių nesiskyrė. Analizuojant $CD8^i$ limfocitus nepasiekusius galutinės

diferenciacijos stadijos ir turinčius tiek CD57, tiek CD27 paviršines molekules, pastebėta $CD8^{i}CD57^{+}CD27^{+}$ limfocitų kiekio sumažėjimo tendencija 2pSS grupėje. Skirtumų lyginant 1pSS grupės rezultatus su kontrolinės ir 2pSS grupės rezultatais nenustatyta. Statistiškai reikšmingas absoliutaus skaičiaus sumažėjimas 1pSS ir 2pSS grupių pacientų periferiniame kraujyje lyginant su kontrolinės grupės pacientais nustatytas tik tiriant ankstyvos diferenciacijos stadijos $CD8^{i}CD57^{-}CD27^{+}$ T limfocitų populiaciją. Manome, kad šios populiacijos limfocitų kiekio sumažėjimas įtakoja ir $CD3^{+}$ T limfocitų ir $CD8^{+}$ T limfocitų absoliutaus skaičiaus sumažėjimą pSS sergančių pacientų periferiniame kraujyje. Šios populiacijos sumažėjimo priežastims nustatyti reikalingi papildomi detalūs tyrimai.

NK ląstelės dėl savo citotoksinių ir reguliacinių savybių yra viena iš autoimuninio proceso prevencijos grandžių. Literatūroje pateikiami duomenys rodo, kad sergančiųjų autoimuninėmis ligomis NK ląstelių kiekis periferiniame kraujyje yra sumažėjęs, taip pat stebimas sumažėjęs jų funkcinis aktyvumas. Šio tyrimo rezultatai parodė statistiškai patikimą NK ląstelių absoliutaus skaičiaus sumažėjimą 1pSS grupės pacientų kraujyje, lyginant su kontrolinės grupės rezultatais, o lyginant kontrolinės ir 2pSS grupės rezultatus patikimo skirtumo nenustatyta. Analizuojant CD19 žymenį turinčių B limfocitų populiacijos pokyčius, pSS sergančių pacientų periferiniame kraujyje statistikai patikimų skirtumų nenustatyta.

Humoraliniai biožymenys atlieka svarbų vaidmenį diagnozuojant sistemines autoimunines ligas, įvertinant šių ligų aktyvumo stadijas, numatant gydymo strategijas ir tolimesnio ligos vystimosi prognozes. Analizavome keletą tokių biožymenų (BAFF (B ląstelių aktyvavimo faktorius), imunoglobulinų laisvos κ ir λ lengvos grandinės, komplemento komponentas Cd4, bendras IgG, interleukinai IL-27 ir IL-35) pSS sergančiųjų tiriamųjų periferiniame kraujyje.

Ištyrus pacientų periferinio kraujo serumo mėginius nustatytas statistiškai patikimas BAFF koncentracijos padidėjimas 2pSS grupės pacientų kraujo serume, rezultatus lyginant su kontrolinės grupės rezultatais, statistiškai reikšmingo skirtumo tarp pSS pacientų grupių nenustatyta. Patikimo BAFF koncentracijos serume skirtumo tarp 1pSS grupės pacientų ir kontrolinės grupės rezultatų nenustatyta.

Įvertinus imunoglobulinų laisvų lengvųjų κ ir λ grandinių koncentraciją tiriamųjų periferiniame kraujyje, nustatėme, kad, šių molekulių koncentracija pSS pacientų serumo mėginiuose yra padidėjusi. 1pSS grupės pacientų serume nustatytas κ grandinių koncentracijos padidėjimas, tačiau skirtumas nėra patikimas. Patikimas κ grandinių koncentracijos padidėjimas nustatytas 2pSS grupės pacientų serume, lyginant tiek su kontrolinės grupės, tiek ir su 1pSS grupės tiriamųjų rezultatais. Lengvųjų λ grandinių koncentracijos statistiškai patikimas padidėjimas nustatytas 2pSS grupės pacientų serume, lyginant su kontrolinės grupės pacientų rezultatais.

Sergančiųjų pSS grupių ir kontrolinės grupės tiriamųjų kraujo serume esanti C4d koncentracija reikšmingai nesiskyrė. Padidėjęs C4d kiekis nurodomas kaip ūmi ligos stadija, o sumažėjęs kiekis – būdingas sunkesnėse ligos stadijose, nes susijęs su auto-antikūnų gamyba ir sisteminiais ekstra-liaukiniais ligos požymiais. Manome, kad šis rodiklis gali būti daugiau informatyvus vertinant individualiai pacientus, ypač lyginant su kitais klinikiniais rodikliais.

Tyrimo rezultatai parodė, kad bendro IgG koncentracija 1pSS ir 2pSS grupių pacientų kraujo serume buvo statistiškai reikšmingai padidėjusi lyginant su kontrolinės grupės tiriamųjų rodikliais. Patikimo rezultatų skirtumo tarp 1pSS ir 2pSS grupių nenustatėme.

Šie humoralinių veiksnių tyrimo rezultatai, rodo patogenezės mechanizmų skirtumus 1pSS ir 2pSS grupėse, taip pat, kad didesnis B ląstelių homeostazės sutrikimas stebimas 2pSS grupėje.

Pastaraisiais metais tiriant autoimuninių ligų modelius labai susidomėta IL-27 ir IL-35 reguliaciniu poveikiu autoimuniniams procesams.

IL-27 dėl savo pleotropinio veikimo svarbus Th limfocitų vystymuisi/diferenciacijai ir funkcijoms. IL-27 veikia naivių T limfocitų proliferaciją ir inicijuoja Th1 tipo imuninio atsako formavimąsi, slopinant diferenciaciją į Th2 ir Th17 limfocitus. IL-27 turi ir priešuždegiminį poveikį, nes gali slopinti efektorinių ir atminties T limfocitų citokinų sekreciją, tačiau IL-27 gali būti ir kaip patogeninis faktorius, jis skatina anti-Ig ir anti-CD40 antikūnais aktyvuotų B limfocitų ir gemalinių centrų B limfocitų proliferaciją. Šiame darbe buvo pirmą kartą tirta IL-27 raiška pSS pacientų serume, ir nustatytas reikšmingas šio

interleukino koncentracijos padidėjimas pSS pacientų serume rodo, kad jis dalyvauja pSS patogeneziniuose mechanizmuose. Reikėtų detalesnių tyrimų įvertinant kokį vaidmenį, apsauginį ar patogeninį, šis interleukinas vaidina pSS patogenezėje.

IL-35 savo funkcijomis labai panašus į IL-27. Šį interleukiną išskirtinai gamina tik T_{reg} limfocitai, T efektoriniai limfocitai jo neekspresuoja. Šio interleukino poveikis dvejopas, esant ūmiai infekcijai jis gali aktyvinti efektorines ląsteles, o pasireiškiant aktyviai uždegimo stadijai – selektyviai supresuoti efektorines ląsteles indukuojant Treg limfocitus ir taip apsaugant nuo autoimuninio atsako išsivystymo. Iki šiol šio interleukino raiška sergantiesiems pSS nebuvo tirta. Literatūroje nėra duomenų apie šio interleukino vaidmenį pSS patogenezėje, tačiau šiame darbe nustatytas jo kiekio padidėjimas pSS pacientų kraujo serume rodo, kad jis taip pat atlieka tam tikrą vaidmenį šiame patologiniame procese.

CURRICULUM VITAE

Name, last name:	Gintaras Sūdžius
Date of birth:	1983-01-02
Office address:	State Research Institute Centre for Innovative Medicine Žygimantų 9, Vilnius tel. (+370 5) 2469247
E-mail:	g.sudzius@imcentras.lt

Education

2001-2005:	BSc, Biology (Molecular Biology), Vilnius University, Lithuania
2005-2007	MSc, Biology (Medicine Biology), Vilnius University, Lithuania
2008-2012	PhD Studies, Immunology, Institute of Immunology (after reorganization State Research Institute Centre for Innovative Medicine)

Employment:

2008-2008:	Main biologist at UAB “STALMA”, Vilnius, Lithuania
2008-present	Junior research fellow, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania

ACKNOWLEDGMENTS

I would like thank all the people who have helped me during the course of this work. In particular I would like to thank:

- My supervisor dr. A. Šiaurys and dr. I. Dumalakienė for their patient, guidance and encouragement
- My co-workers for theoretical and practical help, for critically reviewing this thesis and for providing valuable comments
- My all colleagues for criticism and priceless advices in writing this thesis
- My family and friends for their constant support, encouragement and understanding

This research was supported by:

- grants No. MIP - 96/2010 and No. LIG – 23/2010 from the Research Council of Lithuania
- Research Council of Lithuania funded program "Competitive financing of short-term research visits"