"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST

DOCTORAL SCHOOL

MEDICINE FIELD

Ph.D. THESIS

SUMMARY

PREDICTIVE FACTORS OF ORGAN DAMAGE IN PATIENTS WITH PRIMARY SJÖGREN SYNDROME

Ph.D. supervisor:

PROF. UNIV. DR. Ruxandra IONESCU

Ph.D. student:

Ancuța MIHAI

BUCHAREST

2023

Table of contents

List of published articles and presentations in scientific events

Journal Papers:

First Author

[J1] Mihai A, Caruntu C, Jurcut C, Blajut F C, Casian M, Opris-Belinski D, Ionescu R, Caruntu A. The Spectrum of Extraglandular Manifestations in Primary Sjögren's Syndrome. *J. Pers. Med.* **2023**, *13*, 961, https://doi.org/10.3390/jpm13060961. **[Impact Factor: 3.508- Q2] (Chapter 1-2).**

[J2] Mihai A, Caruntu A, Opris-Belinski D, Jurcut C, Dima A, Caruntu C, Ionescu R. The Predictive Role of Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Monocytes-to-Lymphocyte Ratio (MLR) and Gammaglobulins for the Development of Cutaneous Vasculitis Lesions in Primary Sjögren's Syndrome. *J. Clin. Med.* **2022**, *11*, 5525, https://doi.org/10.3390/jcm11195525. **[Impact Factor: 4.964 - Q2] (Chapter 3).**

[J3] Mihai A, Chitimus DM, Jurcut C, Blajut F C, Opris-Belinski D, Caruntu C, Ionescu R, Caruntu A. Comparative Analysis of Hematological and Immunological Parameters in Patients with Primary Sjögren's Syndrome and Peripheral Neuropathy. *J. Clin. Med.* **2023**, *12*, 3672, https://doi.org/10.3390/jcm12113672. **[Impact Factor: 4.964- Q2] (Chapter 4).**

[J4] Serban A[#], **Mihai A[#],** Dima A, Balaban DV, Jinga M, Jurcut C. The impact of the COVID-19 pandemic on patients with primary Sjögren syndrome. Rheumatol Int. 2021 Nov;41(11):1933-1940, https://doi.org/10.1007/s00296-021-04967-4, Epub 2021 Aug 28, PMID: 34453578, PMCID: PMC8397857. ([#]Contributed equally) **[Impact Factor: 3.58- Q3] (Chapter 5).**

Co-Author

[J5] Casian M, Jurcut C, Dima A, **Mihai A**, Stanciu S, Jurcut R. Cardiovascular Disease in Primary Sjögren's Syndrome: Raising Clinicians' Awareness. Front Immunol. 2022 Jun 9;13:865373, https://doi.org/10.3389/fimmu.2022.865373, PMID: 35757738; PMCID: PMC9219550. **[Impact Factor: 8.786- Q1] (Chapter 2)**

[J6] Balaban DV, **Mihai A**, Dima A, Popp A, Jinga M, Jurcut C. Celiac disease and Sjögren's syndrome: A case report and review of literature. World J Clin Cases. 2020 Sep 26;8(18):4151-4161, https://pubmed.ncbi.nlm.nih.gov/33024773/, PMID: 33024773; PMCID: PMC7520766. **[Impact Factor: 1.823- Q4] (Chapter 2)**

Conference Papers:

[C1] Mihai A, Mardale D, Opris-Belinski D, *et al* SAT0220 BIOLOGICAL PREDICTORS OF ECHOGRAPHIC SALIVARY GLAND INVOLVEMENT SEVERITY IN PATIENTS WITH SJÖGREN'S SYNDROME, *Annals of the Rheumatic Diseases* 2020;**79:**1053, http://dx.doi.org/10.1136/annrheumdis-2020-eular.2292. **(Chapter 2)**

[C2] Mihai A, Mardale D, Opris-Belinski D, *et al* AB0429 NEUTROPHIL TO LYMPHOCYTE RATIO INDEPENDENTLY PREDICTS CUTANEOUS MANIFESTATIONS IN PATIENTS WITH SJÖGREN'S SYNDROME, *Annals of the Rheumatic Diseases* 2020;**79:**1513-1514, http://dx.doi.org/10.1136/annrheumdis-2020-eular.2296. **(Chapter 3)**

[C3] **Mihai A**, Diana Maria C, Jurcut C, *et al* AB0660 PREDICTIVE PARAMETERS FOR THE DEVELOPMENT OF PERIPHERAL NEUROPATHIES IN PRIMARY SJÖGREN'S SYNDROME, *Annals of the Rheumatic Diseases* 2023;**82:**1532-1533, http://dx.doi.org/10.1136/annrheumdis-2023-eular.733. **(Chapter 4)**

[C4] **Mihai A**, Diana Maria C, Jurcut C, *et al* AB0600 THE FOLLOW-UP OF pSS PATIENTS WITH PERIPHERAL NEUROLOGIC INVOLVEMENT IN A TERTIARY CENTER, *Annals of the Rheumatic Diseases* 2023;**82:**1501, doi/10.1136/annrheumdis-2023-eular.734. **(Chapter 4)**

[C5] **Mihai A**, Adriana Elena N, Jurcut C, *et al* AB0599 CLINICAL AND SEROLOGICAL CHARACTERISTICS OF SERONEGATIVE PRIMARY SJÖGREN'S SYNDROME PATIENTS, *Annals of the Rheumatic Diseases* 2023;**82:**1500, https://doi.org/10.1136/ard.61.6.554. **(Chapter 4)**

Introduction

Presentation of the field of the doctoral thesis

In recent years, there has been an increase in interest in the role of hematological indicators in the assessment of autoimmune disease activity and their correlation with several disease complications. Identifying and evaluating the role of hematological and immunological parameters in predicting the occurrence of extraglandular manifestations (EGMs) and their complications in primary Sjögren syndrome (pSS) patients, is necessary. Therefore, the identification of the cut-off value of predictive parameters that correlated with the development of complications in pSS patients is less studied. As well, the severe impact of the severe acute respiratory syndrome coronavirus (SARS-CoV-2) pandemic on the entire population, as well as the sequelae left in all domains, have an even greater impact on autoimmune diseases patients, and with pSS respectively.

Scope of the doctoral thesis

The thesis aim is to identify the role of hematological and immunological parameters in predicting the development of extraglandular cutaneous and neurologic manifestations and their complications in patients diagnosed with pSS. Besides these, we managed to investigate the perspective of pSS patients regarding various aspects of the disease during the SARS-CoV-2 outbreak, including both the impact of coronavirus disease 2019 (COVID-19) on the disease itself and the effects of vaccination against SARS-CoV-2.

Content of the doctoral thesis

Chapter 1 presents general aspects of primary Sjögren syndrome. *Chapter 2* describes the EGMs of pSS, regarding their physiopathology, diagnosis, and treatments. *Chapter 3* includes the first research study which evaluated the cutaneous manifestations types developed during the follow-up period followed by the identification of reliable and easily accessible hematological predictive elements for the development of cutaneous vasculitis lesions (CVL), in pSS patients. *Chapter 4* exposes our aim to evaluate and identify hematologic and immunologic parameters and their predictive potential for peripheral neuropathy development in pSS patients. *Chapter 5* refers to the third research study, where pSS patients' perspectives on the general aspects of the disease and on overall life during the SARS-CoV-2 outbreak, the impact of COVID-19 on disease symptoms and the impact

of vaccination against SARS-CoV-2 were evaluated. *Chapter 6* covers thesis contributions and future perspectives.

1. Primary Sjögren Syndrome

1.1 Introduction

Primary Sjögren Syndrome is a systemic chronic autoimmune rheumatic disorder of unknown etiology, characterized by lymphocytic infiltration with immune-mediated destruction of exocrine glands, primarily including salivary and lacrimal glands (1). In this pathology any organ system may be involved, expressed in various and complex clinical EGMs.

1.2 Pathogenic mechanisms

The interaction between genetic and environmental factors is thought to play a crucial role in susceptible individuals, leading to the dysregulation of the immune system and pSS development (2). The function of specific cytokines and chemokines and their expression by cells of the innate and adaptive immune systems are actively involved in pSS pathogenesis, including extraglandular involvement (3, 4).

Epstein–Barr virus (EBV) has a well-established tropism for B cells, favoring the development of lymphoproliferative processes, the most severe extraglandular complication in pSS (5). Based on genome-wide studies, key steps in pSS triggering were identified, such as aberrant activation of the innate immune response, through the IFN and NF-kB pathways, atypical recruitment to lymphoid sites, and T-cell activation with HLA susceptibility ascending (6). The Th17 cells play a fundamental role in maintaining mucosal barrier integrity. In pSS, Th17 cells produce IL-17 and other inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-22, and IL-26, inducing and mediating pro-inflammatory responses (7). Dysregulation of B lymphocytes also plays a key role in autoimmunity processes, particularly in lymphoma development (8, 9). B cells of pSS patients may secrete cytokines, such as IL-6 and IL-10 (10), and also may produce a B cell activating factor (BAFF) (11). In pSS patients with EGMs that developed lymphoid proliferations was observed a tendency to high BAFF levels (12). DCs can be activated by a Toll-like receptor (TLR), leading to the production of type I interferon (IFN) (13), which induces the production of the BAFF. As well, in pSS patients with EGMs, a higher number of NK cells was detected, implying their role in clinically aggressive disease (14).

1.3 Diagnostic and treatment

The classification criteria for pSS were published in 2016 by ACR/EULAR (American College of Rheumatology/European League against Rheumatism) (15). These classification criteria apply to any patient with symptoms of ocular or oral dryness according to American European Consensus Group criteria (AECG) questions or to patients with the positivity of at least one of the domains of the EULAR Sjögren's syndrome disease activity index (ESSDAI) questionnaire. The recommendations provide only therapies for symptom relief and not a target-organ type approach for systemic manifestations. To date, no biologic drugs have been approved by the regulatory agencies, the Food and Drug Administration (FDA) or the European Medicines Agency (EMA), for the treatment of pSS.

2. Extraglandular manifestations of primary Sjögren syndrome

During disease progression, most pSS patients will develop EGMs (Figure 2.1) The effective management of EGMs implies an early diagnosis, if possible before their clinical expression by means of predictive biomarkers, efficient and accurate investigation tools and scores, as well as personalized treatments, aiming to prevent complications and improve the patient's quality of life.

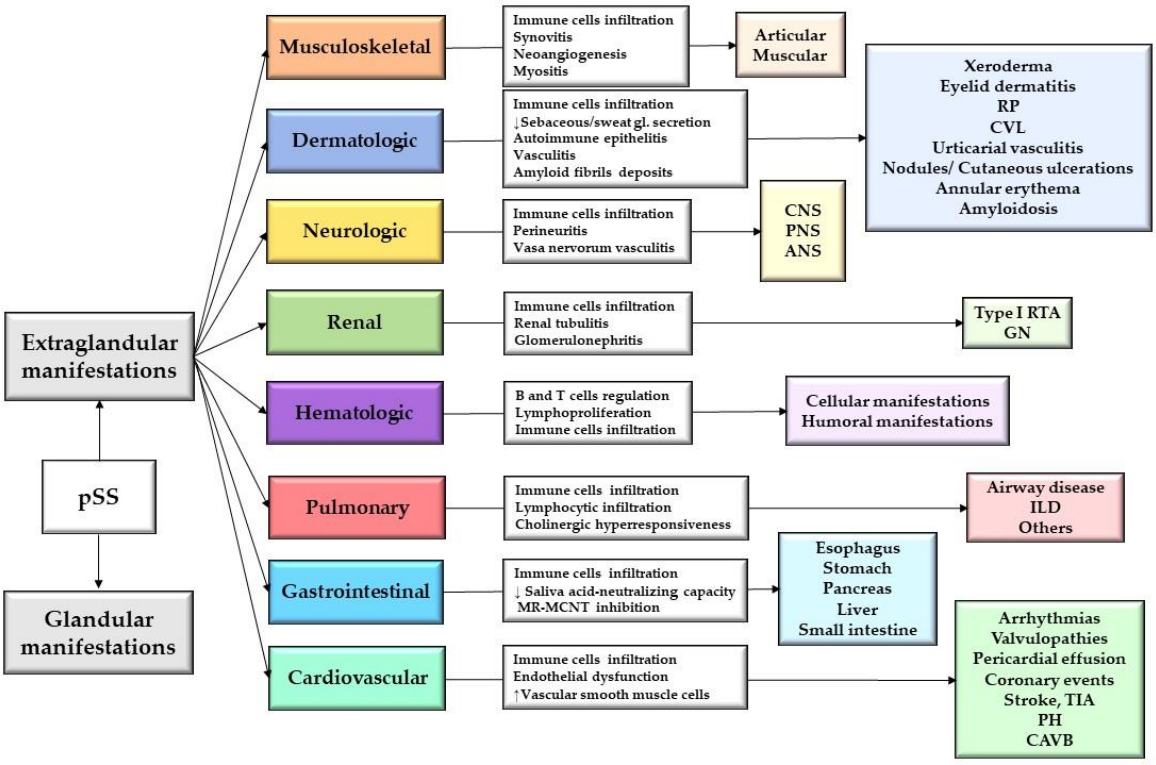


Figure 2.1 Extraglandular manifestations in pSS.

Abbreviation: RP, Raynaud phenomenon; CVL, cutaneous vasculitis lesions; CNS, central nervous system; PNS, peripheral nervous system; ANS, autonomic nervous system; RTA, renal tubular acidosis; GN, glomerulonephritis; ILD, interstitial lung disease; MR- MCNT, inhibit muscarinic receptor-mediated cholinergic neurotransmission; TIA, transient ischemic attacks; PH, pulmonary hypertension; CAVB, congenital atrioventricular block. ↓, low; ↑, high.

2.1 Dermatological manifestations

A large spectrum of skin manifestations may be present in patients with pSS, from common xeroderma to severe vasculitis, including other rare associated conditions (16). Cutaneous vasculitis lesions (CVL) have been reported in 10–30% of pSS patients and are

considered the most clinically and prognostically significant cutaneous complication in this pathology (88).

The CVL pathophysiology presents aspects of leukocytoclastic vasculitis, characterized by fibrinoid necrosis of the vessel walls, leukocytosis and extravasation of erythrocytes, and the presence of IgM, IgG, and C3 around the vessel (89, 109). The diagnosis of cutaneous manifestations is mostly clinical while skin biopsy is recommended in complex cases. Treatment of cutaneous manifestations in pSS patients varies from local emollients in the xeroderma to systemic immunosuppression in CVL, such as glucocorticoids, azathioprine, methotrexate, cyclophosphamide (CYC), or rituximab.

2.2 Neurologic manifestations

Neurologic manifestations may involve the central nervous system (CNS), with a prevalence of around 5% (115), or the peripheral nervous system (PNS), with an incidence between 3.7% to 16% in pSS patients (116-118). The most common patterns are pure sensory polyneuropathies and sensorimotor neuropathies, respectively (118, 119). Vasculitis of the vasa nervorum was described, with lymphocytic, macrophage, and T cell infiltration, as well as necrotizing vasculitis and anti-neuronal antibodies, according to the type of nerve involved (117). Moreover, perineurial infiltration was observed on nerve biopsies of patients with sensorimotor neuropathy (126).

Clinical neurologic symptoms and signs, electromyographic results, and nerve biopsy are the main elements in the diagnosis of peripheric neuropathy (128). As first-line treatment for neuropathic pain, is tricyclic antidepressants, followed by serotonin-norepinephrine reuptake inhibitors, intravenous immunoglobulins, mycophenolate mofetil (120, 141, CYC and anti-BLyS/BAFF therapy, belimumab in ongoing studies (120, 143).

The other EGMs, such as pulmonary, hematological, gastrointestinal, renal, musculoskeletal and cardiovascular are equally important and complex.

3. Study I

The predictive role of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocytes-to-lymphocyte ratio (MLR) and gammaglobulins for the development of cutaneous vasculitis lesions in primary Sjögren's syndrome

3.1 Introduction

Cutaneous involvement in pSS is relatively common and various manifestations may be present. The most clinically and prognostically significant cutaneous complications associated with pSS are CVL (17).

3.2 Objectives

The aim of this study was to identify reliable and easily accessible hematological and immunological elements and their predictive role in cutaneous manifestations development in pSS.

3.3 Materials and Methods

This is a retrospective study involving 245 subjects between April 2015 and November 2021, of which 124 were patients diagnosed with pSS and 121 were control subjects. At the initial visit, a thorough general assessment was conducted, recording all glandular and extraglandular disease expression, blood sample results, skin biopsies, and disease activity scores. Control subjects were selected from patients without any known diseases. Statistical analysis was performed using SPSS software (version 26.0, SPSS, Chicago, IL, USA).

3.4 Results

Lower values for erythrocytes, leukocytes, neutrophils, lymphocytes, monocytes and platelets were detected in the patient's group compared to the control group ($p < 0.001$). Hematological ratios: MLR, PLR and NLR were significantly higher in the pSS patients' group. Gammaglobulins revealed similar differences, with a mean value of 1.45 ± 0.40 g/dL in the pSS group, compared to 1.05 ± 0.16 g/dL in the control group, ($p < 0.001$) (Figure 3.1).

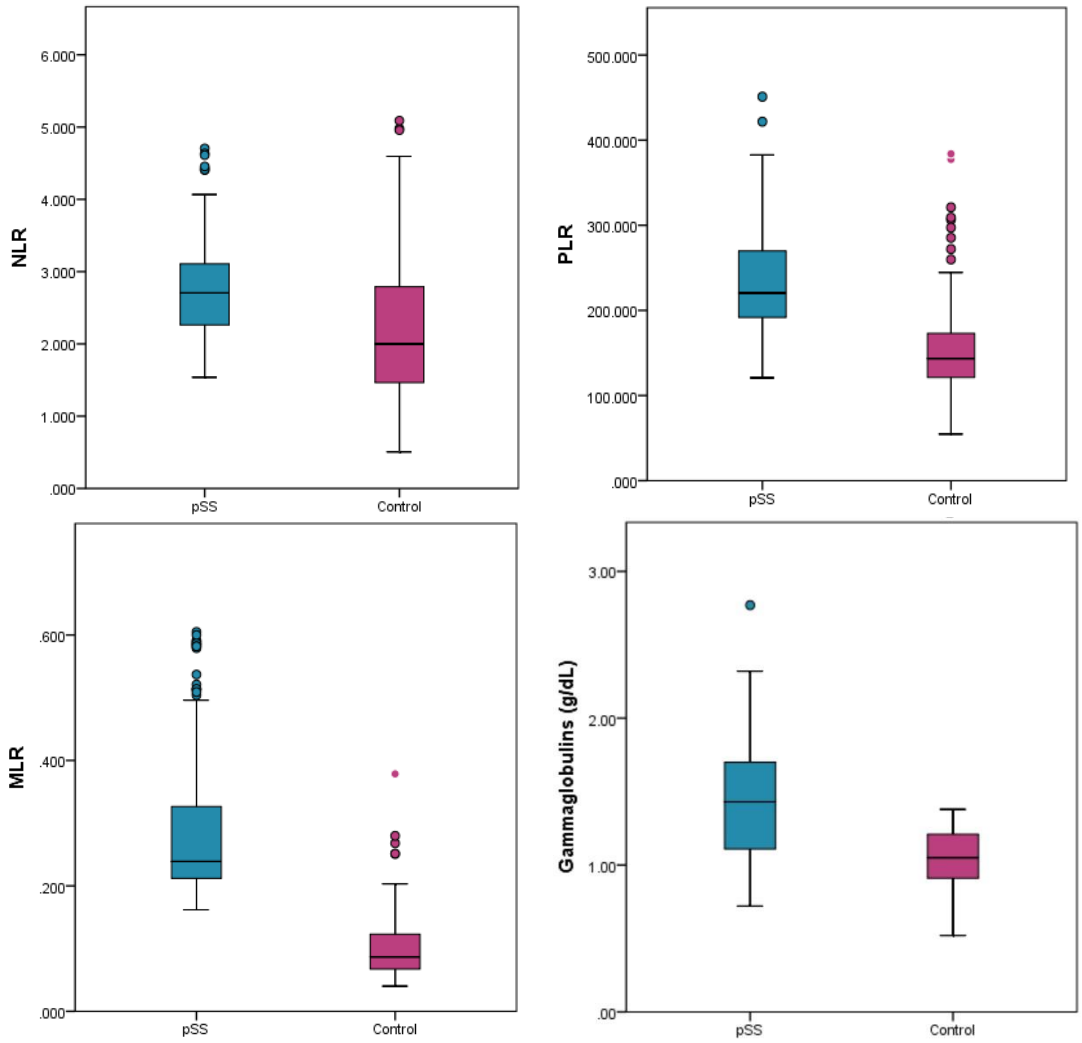


Figure 3.1 Comparison for NLR, PLR, MLR and gammaglobulins between pSS patients and control group.

Of 124 patients, 105 (84.67%) were ANA and anti-Ro/SSA antibodies positive and 76 (61.29%) were anti-La/SSB antibodies positive. Most patients had at the diagnosis moment an ESSDAI score of moderate to severe disease (59.67%), while mild disease activity was detected in 50 patients (40.32%). In bivariate analysis, we observed at the diagnosis moment a positive correlation between increased value of – NLR, PLR, MMLR, gammaglobulins – and ESSDAI and VASp scores.

Cutaneous manifestations (CM) developed in 50 pSS patients (40.32%) (Figure 3.4) and no vasculitis lesions were present at the moment of diagnosis. During the follow-up period, 34 patients (27.41%) have developed CVL and 66 patients (48.38%) developed non-vasculitis lesions. Further, bivariate analysis was conducted, based on the presence of CVL in patients diagnosed with pSS, taking into consideration the hematological parameters determined at the moment of initial diagnosis. Thus, increased NLR values were correlated

with the development of CVL in pSS (r = 0.481, p < 0.001). Similar trends were detected for PLR (r = 0.524, p < 0.001), MLR (r = 0.375, p < 0.001) and gammaglobulins (r = 0.674, p < 0.001).

Using the ROC analysis, the optimal threshold for the three ratios and gammaglobulins was determined, maximizing the composite of specificity and sensitivity for the prediction of cutaneous vasculitis lesions in pSS patients (Figure 3.2).

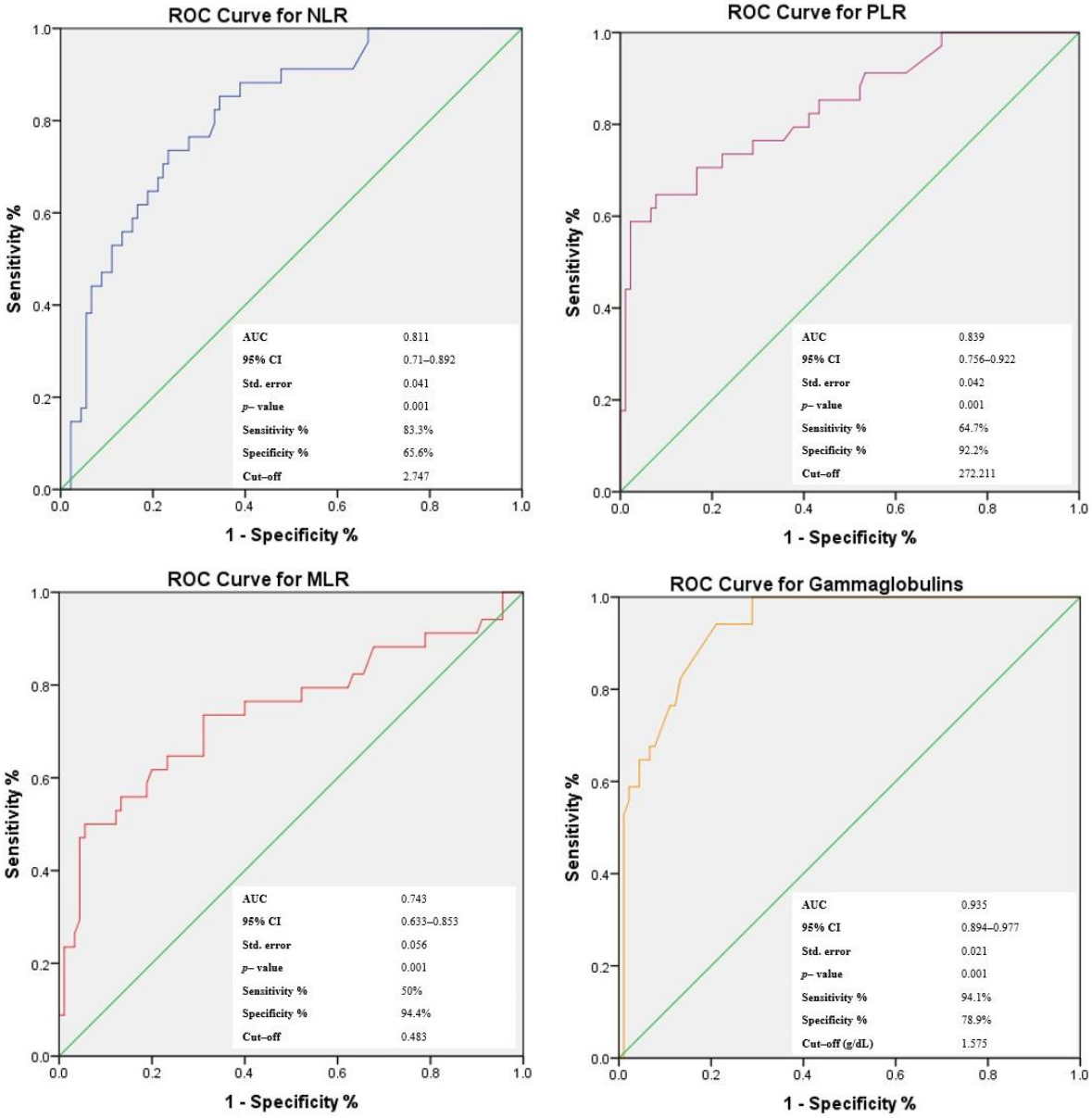


Figure 3.2 Receiver operating characteristic curve of NLR, PLR, MLR nd gammaglobulins levels for the prediction of the cutaneous vasculitis lesions in patients with pSS.

The independent prediction character for the development of cutaneous vasculitis lesions was confirmed using multiple linear regression, for all three hematological ratios – NLR, PLR, MLR – and gammaglobulins, investigated in the study (Table 3.1).

Table 3.1 Multiple linear regression analysis for the development of cutaneous vasculitis lesions in pSS.

Laboratory findings	**Unstandardized coefficients**		**Standardized coefficients**	**T**	***p***	**95.0% Confidence interval for B**	
	B	**Standard error**	**Beta**			**Lower bound**	**Upper bound**
(Constant)	**-1.585**	0.176	-	-9.012	0.000	-1.934	-1.237
NLR	0.142	0.045	0.226	3.156	**0.002 ***	0.053	0.2
PLR	0.002	0.001	0.230	3.064	**0.003 ***	0.001	0.003
MLR	0.510	0.214	0.147	2.380	**0.019***	0.086	0.935
Gammaglobulins (g/dL)	0.555	0.067	0.499	8.291	**0.001***	0.423	0.688
Anti-Ro/SSA	0.000	0.000	0.074	1.247	0.215	0.000	0.001
Anti-La/SSB	$-2.15E^{-5}$	0.000	-0.005	-0.079	0.937	-0.001	0.000
Age	0.000	0.002	0.009	0.154	0.878	-0.004	0.005

Abbreviations: MLR, monocyte to lymphocyte ratio; NLR, neutrophil to lympho1cyte ratio; PLR, platelet to lymphocyte ratio. Bold values indicate statistical significance (*****$p<0.05$).

3.5 Discussions

The analysis of cellular ratios — NLR, MLR and PLR — revealed higher values in pSS patients compared to controls, suggesting that, even though all cellular lines were decreased in pSS patients, the important drop in circulating lymphocytes counts is the main actor to influence these differences, these findings are supported by similar results reported from previous studies (18-20).

The pathophysiology described in leukocytoclastic vasculitis, is an intense infiltration with neutrophils, with extensive necrosis of the cutaneous vascular walls (17). One hypothesis is considered that antibodies against anti-Ro/SSA and anti-La/SSB antigens create immune complexes which precipitate and affect the walls of the blood vessels (21). Furthermore, in skin biopsies collected from pSS patients with cutaneous manifestations, B-cell aggregates were frequently found, suggesting their important role in the pathogenesis of pSS and the development of EGMs, such as CVL (22). These pathophysiological mechanisms might also explain the occurrence of B cell lymphoma in pSS patients with CVL (23).

In SLE, RA, Behçet disease, Takayasu arteritis and SSc, positive correlations were found between NLR, PLR and MLR and the disease activity scores (4, 24-26). In Behçet

disease, NLR was closely related to the development of skin manifestations (24). Other reports suggest an association between MLR and NLR, and vascular and cutaneous manifestations in systemic sclerosis (26). Gammaglobulins were intensely expressed in pSS patients, in accordance with pre-existing studies (19, 27, 28). In our opinion, easily detectable serum elements, which could place the patients into high-risk groups for the emergence of CVL, might prove to be very useful for the early detection of these types of EGMs in pSS, leading to timely and more efficient treatment, using individualized therapeutic strategies for the benefit of the patients.

3.6 Conclusions

NLR, PLR and MLR were increased in pSS patients, positively correlated with CVL and confirmed their independent predictive character for the development of CVL. Additionally, gammaglobulins confirmed their independent prediction character in CVL development. Hence, our results support that these cost-effective and widely available parameters could become valid elements that might be used for the early detection of patients at risk for the development of CVL.

4. Study II

Comparative analysis of hematological and immunological parameters in patients with primary Sjögren's syndrome and peripheral neuropathy

4.1 Introduction

Peripheral neuropathy (PN) is the presenting manifestation in a quarter of pSS patients (29). The most common PN form is distal sensory axonal polyneuropathy, followed by sensorimotor neuropathy (30). The PN type is diagnosed by clinical neurological symptoms and signs, electromyographic assessment and nerve biopsy. The role of hematologic ratios was studied in a wide range of autoimmune diseases and sporadic in pSS (31-33). Previous studies revealed that low levels of complement fractions were correlated with systemic manifestations and disease activity in patients with SLE, vasculitis and myasthenia gravis (25, 34). Gammaglobulins were associated with increased immune activity in many autoimmune diseases (35, 36). Furthermore, reduced concentrations of vitamin D were associated with increased inflammation and high disease activity in SLE, intestinal bowel disease, RA and pSS (37).

4.2 Objectives

This study aimed to evaluate hematologic and immunologic parameters and their predictive potential for PN development in pSS patients.

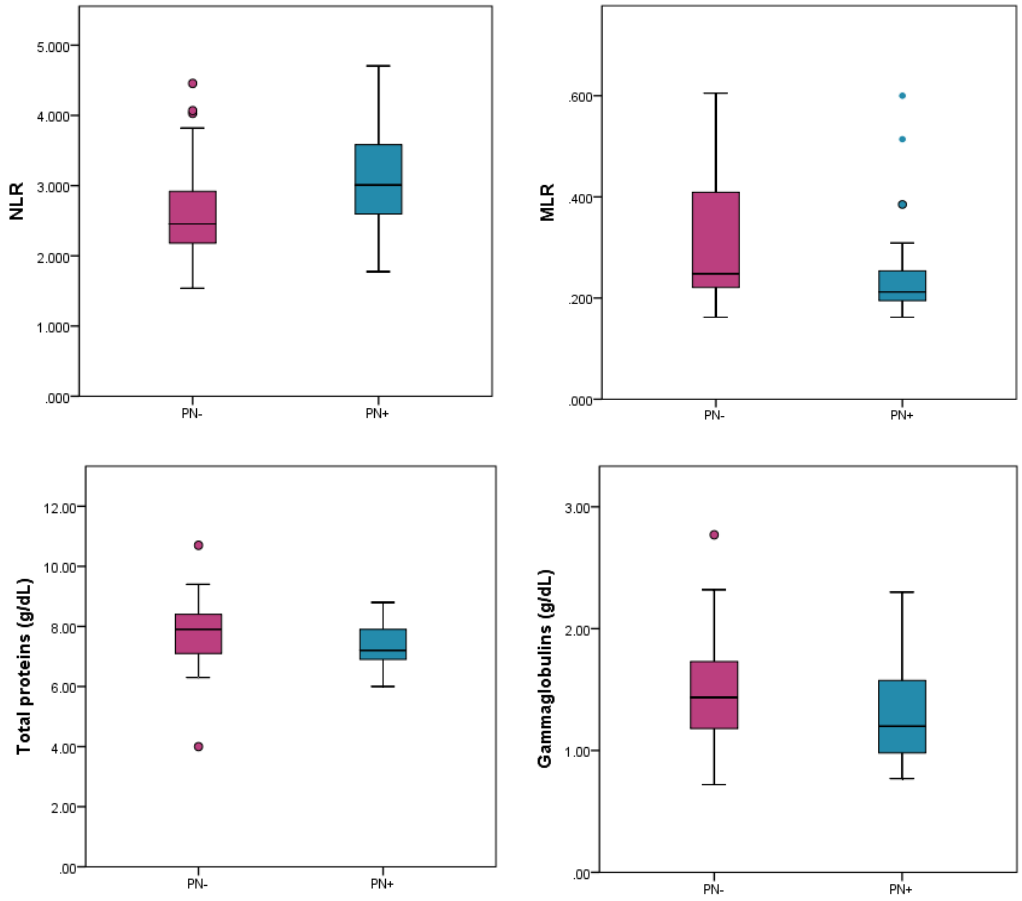
4.3 Materials and methods

We retrospectively analyzed the medical records of pSS patients admitted to our department between April 2015 and December 2021. All newly diagnosed patients were enrolled in a periodical follow-up program, with trimestral visits, to reassess disease progression, including the occurrence of EGMs, and the response to therapy.

Patients who expressed neurological complaints were clinically examined by neurologists, using nerve conduction studies and electrochemical skin conductance. A reduced version of the total neuropathy score (TNSr) was used to evaluate the severity of neuropathy (38). Electrochemical skin conductance was evaluated in patients with symptoms such as tingling, pain and loss of temperature detection, using SUDOSCAN (39). Hematological, immunological and inflammatory markers determined at the disease diagnosis moment were recorded for all the pSS patients of the study.

4.4 Results

During the follow-up period, about a quarter of patients (26%) developed PN. Patients from the PN+ group had significantly higher ESSDAI and VASp scores at the initial visit (p = 0.001). The analysis of cellular ratios revealed that NLR was significantly higher in the PN+ group of patients, with a mean value of 3.14 ± 0.76, compared to 2.58 ± 0.58 in the PN− group (p = 0.001), while MLR was significantly lower in the PN+ group, with a mean value of 0.24 ± 0.96, compared to 0.30 ± 0.12 in the PN− group (p = 0.003). significantly lower baseline mean value for total proteins and gammaglobulins in the PN+ group of patients, compared to PN− group (p = 0.019, p = 0.012, respectively). A similar trend was found for the complement fraction, C4, which had significantly lower values in the PN+ group compared to PN− group (p = 0.001, respectively) (Figure 4.1).



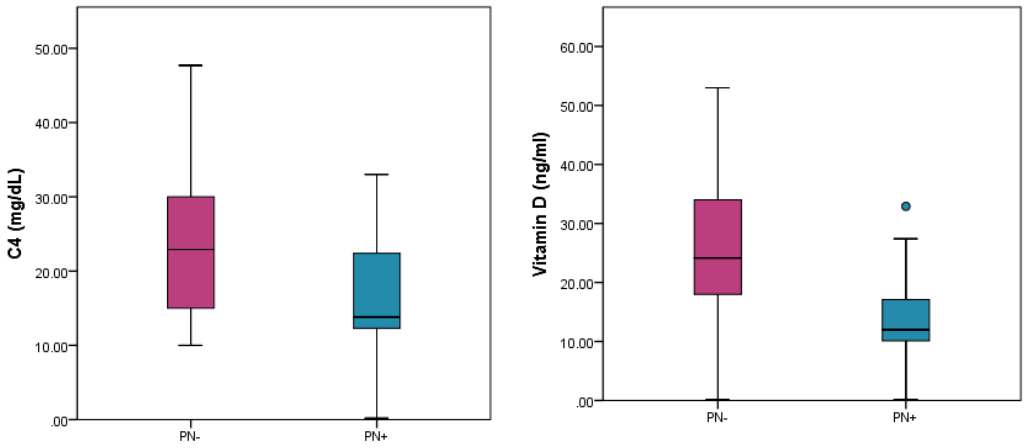
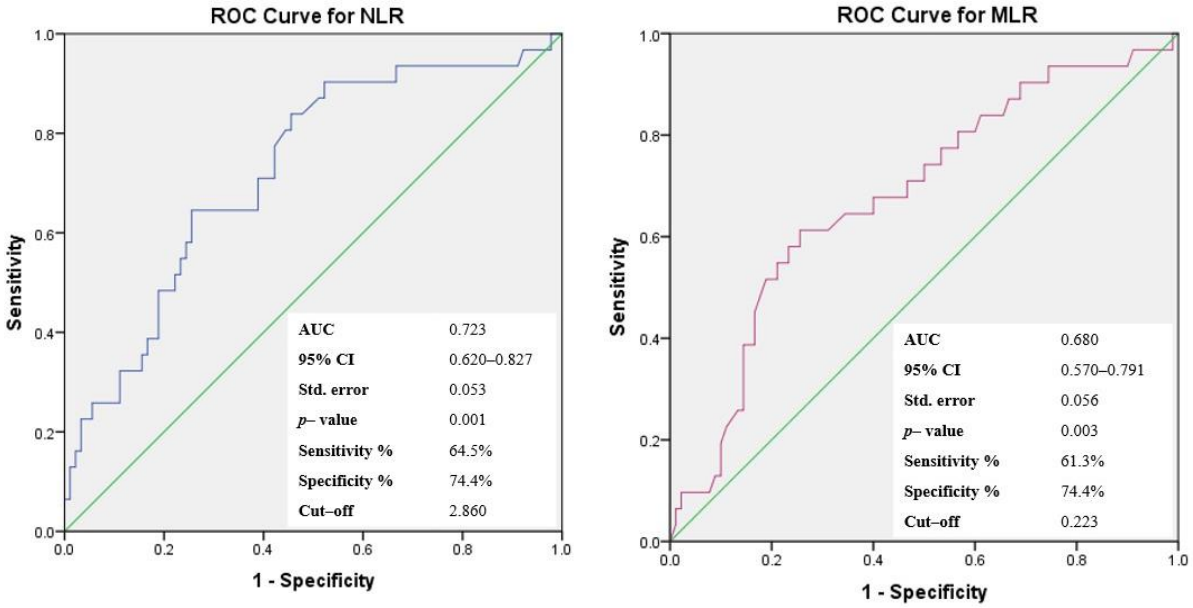


Figure 4.1 Comparison for NLR, MLR, total proteins, gammaglobulins, C3, C4 and Vitamin D between PN- and PN- pSS patients.

We continued the analysis focusing on NLR, MLR, total proteins, gammaglobulins, C4 and vitamin D—, all parameters that revealed statistically significant differences in patients who developed PN during the follow-up period. The ROC curve analysis was used for the prediction of neurological involvement in pSS patients in order to determine the optimal threshold for the variables, maximizing the composite of specificity and sensitivity (Figure 4.2).



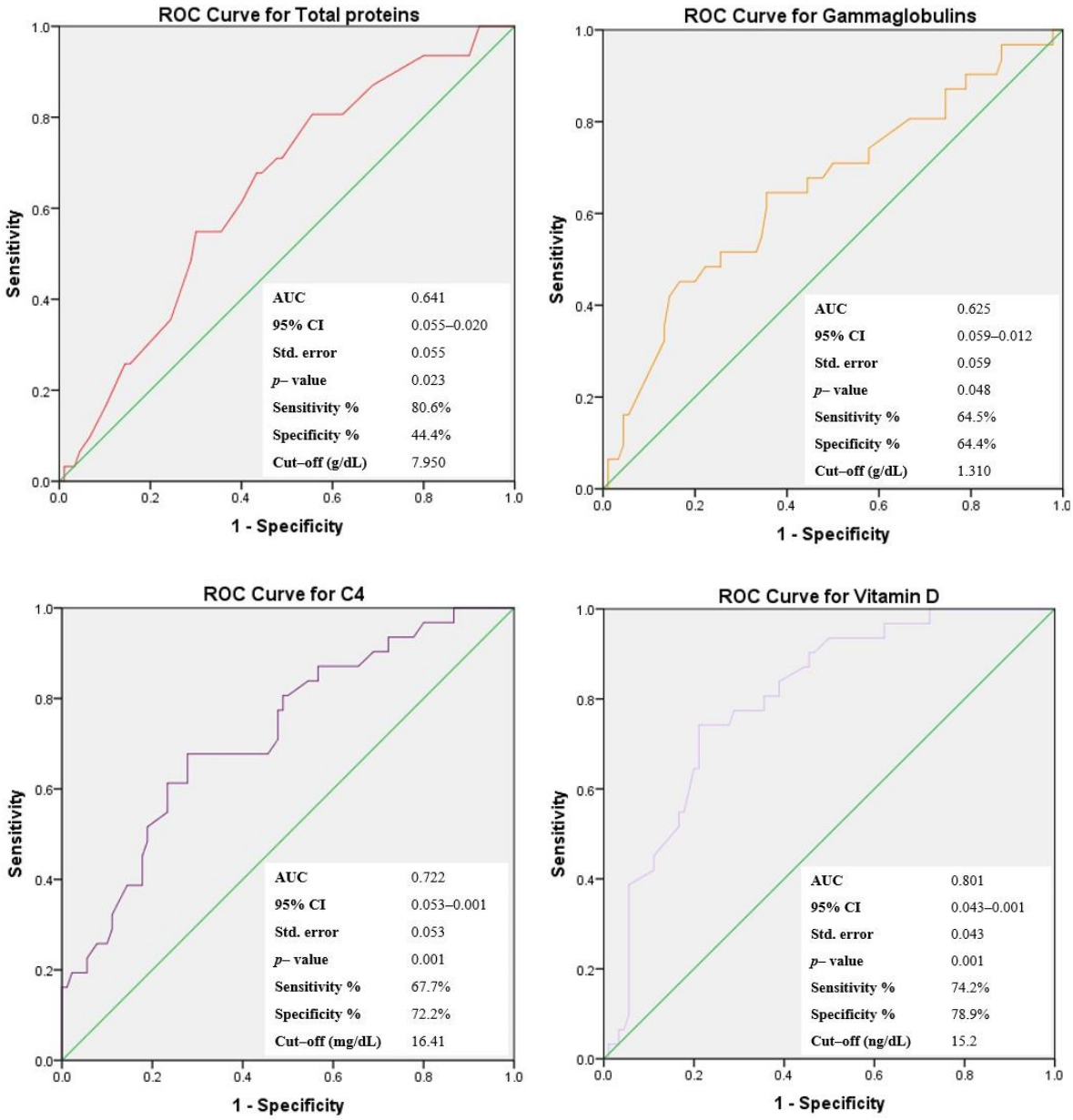


Figure 4.2 Receiver operating characteristic curve of NLR, MLR, total proteins, gammaglobulins, C4 and vitamin D levels for the prediction of the neurologic involvement in pSS patients.

In the multivariate analysis, performed with multiple linear regression, the most relevant hematological and immunological parameters were considered elements that might predict the development of PN in pSS patients. The independent prediction character for the development of PN in pSS patients was confirmed for NLR, MLR, gammaglobulins, C4 and vitamin D (Table 4.1).

Table 4.1 Multiple linear regression analysis for the development of PN in pSS patients.

Laboratory findings	Unstandardized coefficients		Standardized coefficients	T	*p*	95.0% Confidence interval for B	
	B	Standard error	Beta			Lower bound	Upper bound
(Constant)	**0.387**	0.393	-	3.527	**0.001***	0.608	2.166
NLR	0.148	0.058	0.230	2.542	**0.012***	0.033	0.263
MLR	-0.741	0.276	-0.211	-2.680	**0.008***	-1.289	-0.194
Total proteins (g/dL)	-0.066	0.038	-0.133	-1.759	0.081	-0.141	0.008
Gammaglobulins (g/dL)	-0.257	0.085	-0.238	-3.013	**0.003***	-0.426	-0.088
C3 (mg/dL)	0.001	0.001	-0.016	-0.194	0.847	-0.002	0.002
C4 (mg/dL)	-0.009	0.004	-0.186	-2.204	**0.030***	-0.018	-0.001
Vitamin D (ng/mL)	-0.010	0.004	-0.257	-2.657	**0.009***	-0.017	-0.003
Age	0.003	0.003	0.072	0.993	0.353	-0.003	0.008

Abbreviations: NLR, neutrophil to lymphocyte ratio; MLR, monocyte to lymphocyte ratio; C3 and C4, complement 3 and 4.

Bold values indicate statistical significance (*****$p < 0.05$)

4.5 Discussions

The neutrophils act as immune cells in autoimmune diseases, their infiltration being achieved through complex immune mechanisms and complement system-mediated processes (40). In pSS, lymphocytes participate in the intratissular production of auto-antibodies and were associated with an increased risk of lymphoma development due to chronic antigenic stimulation (41, 42). In autoimmune diseases, low circulating levels of monocytes are attributed to their recruitment in the affected tissues (43, 44). In different autoimmune diseases, such as SLE, RA, SSc, polymyositis/dermatomyositis (PM/DM) and pSS, higher values of hematological ratios, NLR and MLR, positively correlated with inflammatory markers and disease activity (19, 20, 45).

The ROC curve analysis revealed the superior predictive power of NLR compared to MLR, with a higher sensitivity at the determined cut-off value, for the early detection of neurological involvement in pSS patients. Hypogammaglobulinemia was reported in different autoimmune diseases, such as SLE and pSS, having various etiologies (46). Complement fixation and consumption may play an essential role in neurologic acute pathophysiology and facilitates neuroinflammation in autoimmune diseases (47, 48). The connection between vitamin D deficiency and peripheral neuropathy development in patients with diabetes, rheumatoid arthritis, SLE (49, 50) and pSS were previously suggested (51).

Different mechanisms were suggested for neurological manifestations in pSS patients, based on the histological and serological findings, such as vasculitis of the vasa nervorum with concomitant lymphocytic and macrophage infiltration, necrotizing vasculitis and anti-neuronal antibodies, according to the type of nerve involved (52, 53). The TNSr score revealed that all pSS patients with neurological manifestations exhibited moderate to severe impairment, while skin conductance, tested with SUDOSCAN, exhibited moderate and severe dysfunction in most of these patients. Similar results were reported in previous studies (54, 55).

4.6 Conclusions

NLR, MLR, gammaglobulins, C4 and vitamin D revealed statistically significant variations, even from the moment of pSS diagnosis, in patients who developed peripheral neuropathy during the follow-up period. These parameters demonstrated that they are predictive in peripheral neuropathy development during the disease evolution in pSS.

5. Study III

The impact of the COVID-19 pandemic on patients with primary Sjögren syndrome

5.1 Introduction

Considering the increasing number of cases and the worldwide distribution, in March 2020, the World Health Organization (WHO) recognized the infection with the new severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV-2) as being pandemic. However, beyond limiting the SARS-CoV-2 infection, these measures had a significant impact on the general quality of life, the access to some specific medications (i.e., HCQ) and on healthcare providers of patients with chronic diseases, including autoimmune pathologies like SS (56, 57).

5.2 Objectives

The aim of this study was to investigate the Romanian pSS patients' perspective on the general aspects of the disease and on overall life during the SARS-CoV-2 outbreak, the impact of COVID-19 on disease symptoms and the impact of vaccination against SARS-CoV-2 in this group of patients.

5.3 Materials and methods

This study was based on an online questionnaire-based survey and the methodology followed the available recommendations (57, 58). The questions were related to the main aspects concerning the SARS-CoV2 outbreak in patients with SS, including the impact of COVID-19 disease and vaccination against the new coronavirus on these patients. The questions were uploaded to a dedicated online platform (https://www.surve ymonkey.com/) and distributed either via direct messaging to patients from our center's cohort or by means of our local pSS patient groups via social media platforms by one of the patient's representatives. Our survey was conducted 1 year after the beginning of the outbreak.

5.4 Results

The number of respondents to the online survey was 137 patients. The impact of the SARS-CoV2 outbreak in patients with SS was expressed by sadder/ depressive and more agitated /anxious states, in 33 patients (24.0%) and 47 patients (34.3%), respectively. Regarding access to healthcare providers, 27 patients (18.7%) postponed the consultation

for fear of getting SARS-CoV2 and 12 patients (8.7%) needed healthcare, but could not access the public hospital services.

In our study group, 31 patients (22.6%) responded that they have had COVID-19. The most persistent symptoms, lasting for weeks or months after the infection, were more severe oral dryness (10 patients, 40.0%) and nasal dryness (32.0%). Among the respondents, 53 patients (41.4%) were vaccinated against SARS-CoV2 with at least one dose. Only three vaccinated patients (10.7%) reported that their dryness-related symptoms were aggravated after the vaccine. The main reason for not getting vaccinated was the fact that the patients were insecure about receiving the SARS-CoV2 vaccine while having a diagnosis of pSS (30 patients; 40.5%).

5.5 Discussions

The general characteristics of the study group (are compatible with similar pSS cohorts. There are few studies evaluating the emotional impact of the SARS-CoV-2 outbreak in patients with SS. In our study, 18.7% of patients postponed the consultation out of fear of infection, compared to the Carubbi et al. study (59) where 95.0% of patients with pSS had their rheumatology consultation cancelled. These discrepant results might be in part due to the fact that the Carubbi et al study was performed in the first phase of the outbreak. The most important symptom during the infection was weakness (84.0%). More than one-third of the patients questioned reported general symptoms like weakness and fatigue as long-lasting post-COVID-19 issues.

Only three of the vaccinated patients (10.7%) reported aggravation of dryness symptoms after the vaccine. One of the most important concerns related to the SARS-CoV2 vaccine in patients with autoimmune disease was the fear of disease fare after the injection. In an online survey-based study, Felten et al. reported three clusters of opinions regarding vaccination in patients with autoimmune diseases and the fear of disease fare was cited among other patients' concerns (36.6% vs 80.8% in patients willing vs unwilling to be vaccinated, $p<0.0001$) (60).

5.6 Conclusions

pSS does not appear to be a risk factor for severe COVID-19, moreover, the vaccination against COVID-19 in our group of patients appeared to be safe. This information will be useful for developing special programs dedicated to SARS-CoV2 infection and vaccination in patients with pSS and other autoimmune diseases.

Conclusions

1. The complexity of the extraglandular manifestations requires the identification of parameters that correlate with organ damage.
2. Of 124 pSS patients with pSS, 40.32% developed cutaneous manifestations during the follow-up period. A positive correlation between higher values of hematological and immunological parameters - NLR, PLR, MLR and gammaglobulins – detected at the diagnosis moment and cutaneous extraglandular manifestations developed during the follow-up period, was demonstrated.
3. From the patients with cutaneous manifestation, 34 (27.41%) developed cutaneous vasculitis lesions during the follow-up period. The hematological and immunological parameters – NLR, PLR, MLR and gammaglobulins – detected at the time of pSS patients diagnosis, demonstrated their predictive role in cutaneous vasculitis lesions development.
4. ESSDAI and VASp scores at the time of pSS patients diagnosis evidenced positive correlations with cutaneous vasculitis lesions, confirming once again that these types of manifestations are more likely to occur in patients with increased disease activity.
5. Of 121 pSS patients with pSS, 25.61% developed polyneuropathy as neurologic extraglandular manifestation during the follow-up period. The hematological and immunological parameters – NLR, MLR, gammaglobulins, C4 and vitamin D – revealed statistically significant variations, even from the moment of pSS diagnosis, in patients who developed peripheral neuropathy during the follow-up period.
6. Higher values for NLR and lower values for MLR, gammaglobulins, C4 and vitamin D detected at the time of pSS patients diagnosis, demonstrated their predictive role in polyneuropathy development during the follow-up period.
7. ESSDAI and VASp scores at the moment of pSS patients diagnosis, were significantly higher in polyneuropathy patients compared to those without further neurological involvement, confirming the increased disease activity from the beginning in these types of manifestations.
8. Approximately a quarter of Romanian patients with pSS had COVID-19 with mild forms of the disease.

9. Romanian patients with pSS did not present an increased risk of developing a severe form of COVID-19. Moreover, vaccination against COVID-19 in our patients group was safe.

Perspectives for future developments

Our results support that these cost-effective and widely available parameters could become valid elements that might be used for the early detection of patients at risk for the development of cutaneous and neurological manifestations in pSS patients.

These biological parameters might become useful tools for clinicians to monitor disease progression and identify potentially severe extraglandular manifestations in pSS patients.

As well the role of these predictive parameters may be evaluated in the other EGMs and their complications in pSS patients.

The results of the third study will be useful for developing special programs dedicated to SARS-CoV2 infection and vaccination in patients with pSS and other autoimmune diseases.

Selective References

1. Chivasso C, Sarrand J, Perret J, Delporte C, Soyfoo MS. The Involvement of Innate and Adaptive Immunity in the Initiation and Perpetuation of Sjögren's Syndrome. International journal of molecular sciences. 2021;22(2).
2. Giannini M, Felten R, Gottenberg JE, Geny B, Meyer A. Inclusion body myositis and Sjögren's syndrome: the association works both ways. Acta Neuropathol Commun. 2022;10(1):152.
3. Ramos-Casals M, Brito-Zerón P, Bombardieri S, Bootsma H, De Vita S, Dörner T, et al. EULAR recommendations for the management of Sjögren's syndrome with topical and systemic therapies. Annals of the rheumatic diseases. 2020;79(1):3-18.
4. Fu H, Qin B, Hu Z, Ma N, Yang M, Wei T, et al. Neutrophil- and platelet-to-lymphocyte ratios are correlated with disease activity in rheumatoid arthritis. Clinical laboratory. 2015;61(3-4):269-73.
5. Houen G, Trier NH. Epstein-Barr Virus and Systemic Autoimmune Diseases. Frontiers in immunology. 2020;11:587380.
6. Khatri B, Tessneer KL, Rasmussen A, Aghakhanian F, Reksten TR, Adler A, et al. Genome-wide association study identifies Sjögren's risk loci with functional implications in immune and glandular cells. Nat Commun. 2022;13(1):4287.
7. Yamagata T, Skepner J, Yang J. Targeting Th17 Effector Cytokines for the Treatment of Autoimmune Diseases. Arch Immunol Ther Exp (Warsz). 2015;63(6):405-14.
8. Kroese FG, Abdulahad WH, Haacke E, Bos NA, Vissink A, Bootsma H. B-cell hyperactivity in primary Sjögren's syndrome. Expert Rev Clin Immunol. 2014;10(4):483-99.
9. Ferro F, Marcucci E, Orlandi M, Baldini C, Bartoloni-Bocci E. One year in review 2017: primary Sjögren's syndrome. Clinical and experimental rheumatology. 2017;35(2):179-91.
10. Nocturne G, Mariette X. B cells in the pathogenesis of primary Sjögren syndrome. Nature reviews Rheumatology. 2018;14(3):133-45.
11. Carrillo-Ballesteros FJ, Palafox-Sánchez CA, Franco-Topete RA, Muñoz-Valle JF, Orozco-Barocio G, Martínez-Bonilla GE, et al. Expression of BAFF and BAFF receptors in primary Sjögren's syndrome patients with ectopic germinal center-like structures. Clin Exp Med. 2020;20(4):615-26.

12. Cornec D, Costa S, Devauchelle-Pensec V, Jousse-Joulin S, Marcorelles P, Berthelot JM, et al. Blood and salivary-gland BAFF-driven B-cell hyperactivity is associated to rituximab inefficacy in primary Sjögren's syndrome. Journal of autoimmunity. 2016;67:102-10.
13. Ainola M, Porola P, Takakubo Y, Przybyla B, Kouri VP, Tolvanen TA, et al. Activation of plasmacytoid dendritic cells by apoptotic particles - mechanism for the loss of immunological tolerance in Sjögren's syndrome. Clin Exp Immunol. 2018;191(3):301-10.
14. Rizzo C, La Barbera L, Lo Pizzo M, Ciccia F, Sireci G, Guggino G. Invariant NKT Cells and Rheumatic Disease: Focus on Primary Sjogren Syndrome. International journal of molecular sciences. 2019;20(21).
15. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. Arthritis & rheumatology (Hoboken, NJ). 2017;69(1):35-45.
16. Orgeolet L, Foulquier N, Misery L, Redou P, Pers JO, Devauchelle-Pensec V, et al. Can artificial intelligence replace manual search for systematic literature? Review on cutaneous manifestations in primary Sjögren's syndrome. Rheumatology (Oxford, England). 2020;59(4):811-9.
17. Argyropoulou OD, Tzioufas AG. Common and rare forms of vasculitis associated with Sjögren's syndrome. Curr Opin Rheumatol. 2020;32(1):21-8.
18. Wei L, Zhifei X, Xiaoran N, Meilu L, Yang L, Yixuan L, et al. Patients with early-onset primary Sjögren's syndrome have distinctive clinical manifestations and circulating lymphocyte profiles. Rheumatology (Oxford, England). 2022;61(2):597-605.
19. Yıldız F, Gökmen O. Haematologic indices and disease activity index in primary Sjogren's syndrome. International journal of clinical practice. 2021;75(3):e13992.
20. Yang Z, Zhang Z, Lin F, Ren Y, Liu D, Zhong R, et al. Comparisons of neutrophil-, monocyte-, eosinophil-, and basophil- lymphocyte ratios among various systemic autoimmune rheumatic diseases. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica. 2017;125(10):863-71.
21. Generali E, Costanzo A, Mainetti C, Selmi C. Cutaneous and Mucosal Manifestations of Sjögren's Syndrome. Clinical reviews in allergy & immunology. 2017;53(3):357-70.

22. Roguedas AM, Pers JO, Lemasson G, Devauchelle V, Tobón GJ, Saraux A, et al. Memory B-cell aggregates in skin biopsy are diagnostic for primary Sjögren's syndrome. Journal of autoimmunity. 2010;35(3):241-7.
23. Retamozo S, Brito-Zerón P, Ramos-Casals M. Prognostic markers of lymphoma development in primary Sjögren syndrome. Lupus. 2019;28(8):923-36.
24. Hammad M, Shehata OZ, Abdel-Latif SM, El-Din AMM. Neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in Behçet's disease: which and when to use? Clinical rheumatology. 2018;37(10):2811-7.
25. Pan L, Du J, Li T, Liao H. Platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio associated with disease activity in patients with Takayasu's arteritis: a case-control study. BMJ open. 2017;7(4):e014451.
26. Yayla ME, İlgen U, Okatan İ E, UsluYurteri E, Torgutalp M, Keleşoğlu Dinçer AB, et al. Association of simple hematological parameters with disease manifestations, activity, and severity in patients with systemic sclerosis. Clinical rheumatology. 2020;39(1):77-83.
27. Hu ZD, Sun Y, Guo J, Huang YL, Qin BD, Gao Q, et al. Red blood cell distribution width and neutrophil/lymphocyte ratio are positively correlated with disease activity in primary Sjögren's syndrome. Clinical biochemistry. 2014;47(18):287-90.
28. Shen L, Suresh L. Autoantibodies, detection methods and panels for diagnosis of Sjögren's syndrome. Clinical immunology (Orlando, Fla). 2017;182:24-9.
29. Sène D, Jallouli M, Lefaucheur JP, Saadoun D, Costedoat-Chalumeau N, Maisonobe T, et al. Peripheral neuropathies associated with primary Sjögren syndrome: immunologic profiles of nonataxic sensory neuropathy and sensorimotor neuropathy. Medicine. 2011;90(2):133-8.
30. Carvajal Alegria G, Guellec D, Mariette X, Gottenberg JE, Dernis E, Dubost JJ, et al. Epidemiology of neurological manifestations in Sjögren's syndrome: data from the French ASSESS Cohort. RMD Open. 2016;2(1):e000179.
31. Soliman WM, Sherif NM, Ghanima IM, El-Badawy MA. Neutrophil to lymphocyte and platelet to lymphocyte ratios in systemic lupus erythematosus: Relation with disease activity and lupus nephritis. Reumatologia clinica. 2020;16(4):255-61.
32. Tezcan D, Körez MK, Gülcemal S, Hakbilen S, Akdağ T, Yılmaz S. Evaluation of diagnostic performance of haematological parameters in Behçet's disease. International journal of clinical practice. 2021;75(10):e14638.
33. Lian L, Xia YY, Zhou C, Shen XM, Li XL, Han SG, et al. Application of platelet/lymphocyte and neutrophil/lymphocyte ratios in early diagnosis and prognostic

prediction in patients with resectable gastric cancer. Cancer biomarkers : section A of Disease markers. 2015;15(6):899-907.
34. Thurman JM, Yapa R. Complement Therapeutics in Autoimmune Disease. Frontiers in immunology. 2019;10:672.
35. Cuadrado MJ, Calatayud I, Urquizu-Padilla M, Wijetilleka S, Kiani-Alikhan S, Karim MY. Immunoglobulin abnormalities are frequent in patients with lupus nephritis. BMC rheumatology. 2019;3:30.
36. Liang M, Liwen Z, Yun Z, Yanbo D, Jianping C. Serum Levels of IL-33 and Correlation with IL-4, IL-17A, and Hypergammaglobulinemia in Patients with Autoimmune Hepatitis. Mediators of inflammation. 2018;2018:7964654.
37. Illescas-Montes R, Melguizo-Rodríguez L, Ruiz C, Costela-Ruiz VJ. Vitamin D and autoimmune diseases. Life sciences. 2019;233:116744.
38. Siao P, Kaku M. A Clinician's Approach to Peripheral Neuropathy. Semin Neurol. 2019;39(5):519-30.
39. Casellini CM, Parson HK, Richardson MS, Nevoret ML, Vinik AI. Sudoscan, a noninvasive tool for detecting diabetic small fiber neuropathy and autonomic dysfunction. Diabetes technology & therapeutics. 2013;15(11):948-53.
40. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nature reviews Immunology. 2018;18(2):134-47.
41. Sène D, Ismael S, Forien M, Charlotte F, Kaci R, Cacoub P, et al. Ectopic Germinal Center-Like Structures in Minor Salivary Gland Biopsy Tissue Predict Lymphoma Occurrence in Patients With Primary Sjögren's Syndrome. Arthritis & rheumatology (Hoboken, NJ). 2018;70(9):1481-8.
42. Ma WT, Gao F, Gu K, Chen DK. The Role of Monocytes and Macrophages in Autoimmune Diseases: A Comprehensive Review. Frontiers in immunology. 2019;10:1140.
43. Sequí-Sabater JM, Beretta L. Defining the Role of Monocytes in Sjögren's Syndrome. International journal of molecular sciences. 2022;23(21).
44. Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nature neuroscience. 2011;14(9):1142-9.
45. Oukka M, Bettelli E. Regulation of lymphocyte trafficking in central nervous system autoimmunity. Current opinion in immunology. 2018;55:38-43.

46. Jamilloux Y, Magy L, Hurtevent JF, Gondran G, de Seze J, Launay D, et al. Immunological profiles determine neurological involvement in Sjögren's syndrome. European journal of internal medicine. 2014;25(2):177-81.
47. Fukami Y, Koike H, Iijima M, Mouri N, Nishi R, Katsuno M. Role of complement components in vasculitic neuropathy associated with systemic lupus erythematosus and rheumatoid arthritis. Muscle & nerve. 2022;66(2):175-82.
48. Durcan L, Petri M. The clinical and serological associations of hypocomplementemia in a longitudinal sle cohort. Seminars in arthritis and rheumatism. 2020;50(5):1081-6.
49. Skalli S, Muller M, Pradines S, Halimi S, Wion-Barbot N. Vitamin D deficiency and peripheral diabetic neuropathy. European journal of internal medicine. 2012;23(2):e67-8.
50. Yesil H, Sungur U, Akdeniz S, Gurer G, Yalcın B, Dundar U. Association between serum vitamin D levels and neuropathic pain in rheumatoid arthritis patients: A cross-sectional study. International journal of rheumatic diseases. 2018;21(2):431-9.
51. Agmon-Levin N, Kivity S, Tzioufas AG, López Hoyos M, Rozman B, Efes I, et al. Low levels of vitamin-D are associated with neuropathy and lymphoma among patients with Sjögren's syndrome. Journal of autoimmunity. 2012;39(3):234-9.
52. Alunno A, Carubbi F, Bartoloni E, Cipriani P, Giacomelli R, Gerli R. The kaleidoscope of neurological manifestations in primary Sjögren's syndrome. Clinical and experimental rheumatology. 2019;37 Suppl 118(3):192-8.
53. Tani J, Liao HT, Hsu HC, Chen LF, Chang TS, Shin-Yi Lin C, et al. Immune-mediated axonal dysfunction in seropositive and seronegative primary Sjögren's syndrome. Annals of clinical and translational neurology. 2020;7(5):819-28.
54. Dudley MT, Borkum M, Basera W, Wearne N, Heckmann JM. Peripheral neuropathy in HIV patients on antiretroviral therapy: Does it impact function? Journal of the neurological sciences. 2019;406:116451.
55. Stewart S, Thomas S, Van Doormaal PT, Höke A. Relation of exercise and pain in patients with idiopathic distal axonal polyneuropathies. Journal of the peripheral nervous system : JPNS. 2020;25(4):388-94.
56. Giardina F, Izzo R, Gattamelata A, Colafrancesco S, Conti F, Priori R. COVID-19 in Italian Sjögren's syndrome patients: a monocentric study. Rheumatology international. 2021;41(1):235-6.
57. Gaur PS, Zimba O, Agarwal V, Gupta L. Reporting Survey Based Studies - a Primer for Authors. Journal of Korean medical science. 2020;35(45):e398.

58. Eysenbach G. Improving the quality of Web surveys: the Checklist for Reporting Results of Internet E-Surveys (CHERRIES). Journal of medical Internet research. 2004;6(3):e34.

59. Carubbi F, Alunno A, Ferri C, Gerli R, Bartoloni E. The Impact of SARS-CoV-2 Outbreak on Primary Sjögren's Syndrome: An Italian Experience. Frontiers in medicine. 2020;7:608728.

60. Felten R, Dubois M, Ugarte-Gil MF, Chaudier A, Kawka L, Bergier H, et al. Vaccination against COVID-19: Expectations and concerns of patients with autoimmune and rheumatic diseases. The Lancet Rheumatology. 2021;3(4):e243-e5.