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# Clinical Characteristics of Distinct Subgroups of Patients with Primary Sjögren's Syndrome, Classified according to Serological Profiles and the Presence of Cutaneous Vasculitis; a Comparative Study

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# Clinical Characteristics of Distinct Subgroups of Patients with Primary Sjögren's Syndrome, Classified according to Serological Profiles and the Presence of Cutaneous Vasculitis; A Comparative Study

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**Abstract:** This study aimed to determine the specific characteristics of extraglandular manifestations especially cutaneous involvement in patients with primary sjögren's syndrome(pSS), focusing on the different clinical and histologic types of SS-associated vasculitis. A retrospective study was conducted and 402 patients (378 women and 24 men) with pSS were analyzed. Patients were categorized into three subgroups based on serological tests. These; 1) quadruple seropositive (positive for anti-Sjögren's-syndrome-related antigen A antibodies (anti-SSA; anti-Ro) and anti-Sjögren's-syndrome-related antigen B antibodies (anti-SSB; anti-La), rheumatoid factor (RF), and antinuclear antibody (ANA)), 2) double seropositive (positive for ANA and anti-SSA/Ro antibodies), 3) quadruple seronegative (negative for ANA, RF, anti-SSA/Ro and anti-SSB/La antibodies). The number of quadruple seropositive patients was 72 (18.6%), double seropositive 174 (43.2%), and quadruple seronegative was 85 (21.1%). The diagnosis age of quadruple seropositive pSS was 42.4±10.8, significantly younger than patients with double seropositive and quadruple seronegative pSS ($p$ = 0.021, $p$=0.112). In terms of organ involvement, salivary gland enlargement, arthralgias, arthritis, Raynaud's phenomenon, lymphadenopathy, vasculitis-purpura, interstitial lung disease, neurological involvement, autoimmune thyroiditis, renal interstitial disease, anemia, leukopenia, hypergammaglobulinaemia and hypocomplementaemia were more common in quadruple seropositive pSS compared to quadruple seronegative ($p$<0.0001). Vasculitis was present in 20 patients. Of these, 15 were leukocytoclastic vasculitis(LCV), 3 were cryoglobulinaemic vasculitis(CV) and 2 were urticarial vasculitis. There were 10 patients have quadruple seropositivity, 5 double seropositive, 2 isolated RF positive, and 3 quadruple seronegativity patients with vasculitis. The results of this study confirm the strong impact of immunological markers on the phenotype of pSS at the time of diagnosis. Immunological patterns play a central role in the phenotypic expression of the disease, even during the initial diagnostic phase, and can guide physicians in designing personalized treatment plans for patients with pSS.

**Keywords:** Primary Sjogren's syndrome; autoantibodies; Vasculitis; hypocomplementaemia; cryoglobulinemia

## 1. Introduction

Sjögren's syndrome (SS) is a systemic chronic autoimmune inflammatory disease that mainly affects exocrine glands by lymphocytic infiltration, resulting in dryness of the eyes and mouth symptoms [1]. SS may occur alone (primary form-pSS) or overlap with another well-defined rheumatic disease (secondary form-sSS), pointing to differences in the pSS and sSS pathology. SS has a worldwide distribution, and its phenotype may vary as a function of geolocation, race, and ethnicity. The clinical presentation of SS is heterogeneous and varies from sicca symptoms to systemic disease [2]. SS is associated with a diversity of autoantibodies as a result of B cell aberrant activation, with anti-Sjögren's-syndrome-related antigen A antibodies (anti-SSA; anti-Ro) and anti-Sjögren's-syndrome-related antigen B antibodies (anti-SSB; anti-La), rheumatoid factor (RF), and antinuclear antibody (ANA) being the most common encountered [3]. Anti-Ro/SS-A antibody is present in 50-75% of SS patients, and in approximately half of them, anti-La/SS-B antibody is also detected and the

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prevalence of ANA and RF was 50-89% and 38-61%, respectively, in the sera of pSS patients [4]. The autoantibodies might lead to the destruction of epithelial cells, causing gland hypofunction and the development of sicca symptoms, and these autoantibodies would also lead to extraglandular manifestations [5]. Many organs other than the exocrine glands may be affected in patients with Sjögren's disease; these include the skin and joints; the lungs, heart, and gastrointestinal tract, including the pancreas and liver; the kidneys, bladder, and gynecologic system; and both the peripheral nervous system and central nervous system [6]. Immunological markers provide prognostic information both in the diagnosis of the disease, predicting the results, and extraglandular manifestations [7]. Previous studies have emphasized the association of anti-Ro/SSA antibodies with the development of extra-glandular manifestations such as vasculitis, lung involvement, nephritis, and risk of lymphoma [8,9]. In a different study, indicated that RF may be associated with serological positivity for anti-Ro and anti-La, as well as with systemic severe disease, pulmonary involvement, renal disease, and corticosteroid use [10].

Vasculitis is one of the most characteristic extraglandular SS manifestations. The spectrum of cutaneous involvement in pSS is broad, yet it remains a relatively understudied area of research. A relationship has been observed between serological abnormalities and cutaneous vasculitis seen in patients with Sjögren's syndrome [11]. Additionally, many studies have shown that the immunological profile at diagnosis may have a significant impact on the occurrence of vasculitis [12,13]. The size and site of the affected vessels in association with the inflammatory process, account for the wide clinical spectrum, varying from limited cutaneous lesions to life-threatening systemic disease [14,15].

The relationship between anti-Ro/SS-A and anti-La/SS-B autoantibody positivity and clinical findings in Sjögren's patients has been further investigated; however, there are few studies in the literature, especially between RF and ANA antibodies and clinical entities. Unfortunately, there are no studies specifically on quadruple antibody positivity. The present study focused on searching for biomarkers and potential therapeutic targets of SS subsets that vary in an autoantibody profile. To this aim we compared clinical and immunologic features of SS patients categorized into 3 serological subgroups: 1) positive for ANA, RF, anti-SSA/Ro and anti-SSB/La antibodies (quadruple seropositive), 2) negative for ANA, RF, anti-SSA/Ro and anti-SSB/La antibodies(quadruple seronegative), and 3) positive for ANA and anti-SSA/Ro antibodies(*double seropositive*).

This study aimed to determine the specific characteristics of extraglandular manifestations especially cutaneous involvement in patients with pSS, with emphasis on the different clinical, histologic types, and serological of SS-associated vasculitis.

## 2. Materials and Methods

### *2.1. Study Design, Setting, and Ethics*

This comparative study was conducted in the Department of Rheumatology Eskişehir Cıty Hospital in Turkey between December 2019 and May 2024. Ethical approval was acquired from the local ethics committee (decision date: 23 May 2024, decision no: 2024/30) and it was carried out according to the ethical standards stated in the Declaration of Helsinki and its amendments. The method and purpose of the study were explained to all participants in detail, and written informed consent was obtained from each participant. Patients were invited for examination every 3 or 6 months, written informed consent was obtained from all participants at the time of application and information was given about the study.

### *2.2. Study Population*

The study population included 402 consecutive pSS patients (378 women and 24 men) who fulfilled the 2016 EULAR/ACR criteria [16]. The clinical characteristics of the patients were retrospectively collected from their medical records. Laboratory information and pathologic reports were obtained from the patient's files in the hospital's electronic database. As anti-Ro/SSA-positive patients fulfilled the EULAR criteria, a minor salivary gland biopsy (MSGB) was not performed. However, MSGB was also performed in some SSA-positive patients who did not fulfill the EULAR criteria and whose sicca symptoms were not prominent. MSGB was performed in all patients with other autoantibody positivity and negativity. The exclusion criteria were as follows: combined head

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and neck radiation, chronic hepatitis C or human immunodeficiency virus infections, prior lymphoproliferative disease, sarcoidosis, graft-versus-host disease, amyloidosis, IgG4-related disease, fibromyalgia and associated systemic autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, mixed connective tissue disease, myopathies, and systemic sclerosis. In all patients, diseases that may cause sSS were excluded by serologic tests and clinical findings. The levels of complements (C3 and C4), erythrocyte sedimentation rate(ESR), C reactive protein(CRP), immunoglobulins (IgG, IgM, and IgA), anti-nuclear antibody (ANA), anti-dsDNA, rheumatoid factor(RF), anti-CCP antibody, the autoantibody-targeted extractable nuclear antigens (ENAs) were detected by using immune blot method which identified 7 different target autoantigens including U1RNP/Sm, Sm, SS-A, Ro52, SS-B, Scl-70, Jo-1 were retrieved from the case records. Patients without sufficient data for diagnosis of SS in the registry were not included in the study. Procedures general data and laboratory and clinical information at the onset of patients with pSS were retrospectively reviewed, including the first presentation and systemic involvements. Clinical data, such as age at diagnosis, disease duration, oral and ocular dryness, and constitutional symptoms, as well as data on joint, pulmonary, kidney, vasculitis, skin, nervous, gastrointestinal tract, and endocrine involvement, were collected. Schirmer's test was considered positive when less than 5 mm of the paper was humid after 5 minutes. A minor salivary gland biopsy was taken from the lower lip by making an incision. Focal lymphocytic sialadenitis in a minor salivary gland biopsy with one or more foci of lymphocytes per 4 mm2 (focus score ≥ 1) was accepted as the histopathological criteria [17].

The presence and titers of the ANAs were determined using an indirect immunofluorescence method with the Hep2 cell line as an antigen and an antibody titer test; the cutoff value for ANA was 1:80; by the values used in our laboratory; anti-Ro/SSA and anti-La/SSB were detected using enzyme immunoassay; while RF of IgM class (normal range < 30 IU/ml) and other autoantibodies were identified by routine laboratory tests. Patients with cytopenias were evaluated haematologically and malignancy; leukemia; drugs; B12; folate; iron deficiency; or anemia of chronic disease were excluded.Leucopenia was defined as white blood cell count <4.00 × 103/mm3; neutropenia (neutrophil <1.5 x 103/mm3); anaemia (haemoglobin concentration <12 g/dl); thrombocytopenia (platelet count <150 x 103/mm3)

In patients with petechiae and purpura, a skin biopsy was performed after the exclusion of drug reactions and xeroderma. To exclude other etiological causes in all patients with vasculitis; previous or current infections, antibiotics used, malignancy status, vaccinations received in the last 6 months, possible etiological factors; antineutrophil cytoplasmic antibodies (ANCA), immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) syphilis serology, streptococcal antibodies, abdomen ultrasound examination, chest radiography, fecal occult blood tests (FOBT), and skin biopsy were performed in all patients. Direct immunofluorescence (DIF) tests for IgA, IgG, IgM, and C3 were analyzed in the skin biopsy. All patients with vasculitis were evaluated for medium and large vessel vasculitis and systemic involvement. All patients were classified according to the 2012 Revised International Chapel Hill Consensus Conference (CHCC) Nomenclature of Vasculitides [18]. Cryoglobulinemic vasculitis was diagnosed when serum cryoglobulins were positive with characteristic clinical features. The diagnosis of urticarial vasculitis is made when long-lasting (more than 24 hours) indurated weals, which may be itchy, painful, or tender, or accompanied by purpura, occur spontaneously or at minor trauma sites.

The patients were three grouped under the following serological profiles: Group 1; positive for ANA, RF, anti-SSA/Ro, and anti-SSB/La antibodies (quadruple seropositive), group 2; negative for ANA, RF, anti-SSA/Ro, and anti-SSB/La antibodies(quadruple seronegative) and group 3; positive for ANA and anti-SSA/Ro antibodies(*double seropositive*).

### *2.4. Statistical Analysis*

All Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 23.0 software (IBM Corp., Armonk, NY, USA). While descriptive values were expressed as numbers (n) and percentages (%) for categorical values, they were expressed as the mean (standard deviation, SD) if normally distributed and as the median (interquartile range, IQR) if not normally distributed. For continuous variables, comparisons of median values were carried out with the Mann-Whitney U-test. Student's t-test was used as a parametric test. The chi-square test and Fisher's exact test were used

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for categorical variables. The statistical significance level was accepted as $p<0.05$ in all comparisons.

## 3. Results

### *3.1. Basic Characteristics of Total pSS Patients Included in the Present Study*

The baseline characteristics of the patients diagnosed with pSS are shown in Table 1. In total, 402 patients with pSS were retrospectively analyzed with a mean age of 54.3 (18-84) years and the patients were predominantly females 378 (94.0%). Among these patients, 78.8% (317/402) were seropositive (with at least one antibody positivity) and 85 (21.1%) were quadruple seronegative. Anti-SSA was presented in 252 (62.6%) patients and anti-SSB in 75 (18.6%) patients. Other autoantibodies including ANA and RF were presented in 284 (70.6% ) and 96 (23.8%) of patients with pSS, respectively. The combined ANA and SSA positivity was 174 (43.2%). The number of quadruple seropositive patients was 72 (18.6%). ANA staining pattern, with the granular pattern at 61%, nuclear at 20%, homogenous at 10%, and centromere at 6%, and other staining patterns at 3%. MSGB was performed on a total of 232 patients and a focus score≥1 was detected in 220 (94.8%) of them.

### *3.2. Comparison of Clinical Characteristics according to the Presence of Antibodies*

Clinical characteristics and comparison of seropositive, seronegative, and double seropositive pSS were shown in Tables 2 and 3. The diagnosis age of quadruple seropositive pSS was 42.4±10.8, significantly younger than patients with double seropositive and quadruple seronegative pSS ($p$ = 0.021, $p$=0.112). When quadruple seropositive pSS was compared with quadruple seronegative, the presence of symptoms related to glandular dysfunction, such as xerostomia (97.2% vs. 94.1%, $p$ = 0.112) and xerophthalmia (94.0% vs. 88.2%, p = 0.142) was more common in quadruple seropositive pSS (Table 2). Similarly, quadruple seropositive pSS was compared with double seropositive, xerostomia (97.2% vs. 92.%, $p$= 0.242) and xerophthalmia (94.0% vs. 90.8%, $p$ = 0.361) were more common in quadruple seropositive pSS (Table 3). However, this similarity was not found to be statistically significant between double seropositive and quadruple seronegative ($p$=0.345 with $p$=0.526) (Table 4). In terms of organ involvement, salivary gland enlargement, arthralgias, arthritis, Raynaud's phenomenon, lymphadenopathy, vasculitis-purpura, interstitial lung disease, neurological involvement, autoimmune thyroiditis, renal interstitial disease, anemia, leukopenia, hypergammaglobulinaemia and hypocomplementaemia were more common in quadruple seropositive pSS compared to quadruple seronegative ($p$<0.0001) (Table 2). Except for salivary gland enlargement, arthralgias, and hypocomplementaemia, other involvements were more frequent in quadruple seropositive individuals than in those with double seropositivity ($p$<0.0001) (Table 3). Similarly, when double seropositive was compared with quadruple seronegative; salivary gland enlargement, arthralgias, arthritis, lymphadenopathy, and interstitial lung disease were more common in double seropositivity($p$<0.0001) (Table 4). Interstitial lung disease was seen in 98.2% (55/56) of seropositive (at least 1 antibody positive) patients, while it was 1.7% (1/56) in seronegative patients (p<0.0001). NSIP accounted for 89.2% of the ILD pattern, and almost all of them were seen in seropositive patients. Neurological involvement was seen in 95.0% (19/20) of seropositive patients, while it was 5.0% (1/20) in seronegative patients (p<0.0001). Autoimmune thyroiditis was present in 86 patients (21.3%), 74.4% of these patients were ANA positive and ANA staining; granular pattern at 84%, homogenous at 10%, nuclear at 6%. Of the autoimmune thyroiditis patients 79 (91.8%) had Hashimoto's disease, 5 had Graves' disease, and 2 had subacute thyroiditis. Moreover, autoimmune thyroiditis was seen in 83.7% (72/86) in seropositive patients, while it was 9.3% (8/86) in seronegative patients (p<0.0001). Cryoglobulinemia, Raynaud's phenomenon, vasculitis, and renal interstitial disease were not observed in any of the quadruple seronegative patients.

Laboratory findings of quadruple seropositive pSS and quadruple seronegative pSS patients were also compared(Table 2**)**. The level of ESR and CRP was lower in seronegative pSS ($p$ = 0.125 vs. p=0.362). Periphery blood parameters including platelet count ($p$ = 0.001), hemoglobin (p<0.0001), leukocyte count (p<0.0001), and lymphopenia (p<0.0001) levels were lower in quadruple seropositive pSS. The level of hypergammaglobulinemia was found to be higher in individuals with quadruple seropositivity compared to those with quadruple seronegative (p<0.0001). No significant differences were shown between quadruple seronegative pSS and quadruple seropositive pSS in other laboratory

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parameters such as level of creatinine, and variables related to liver, renal, and thyroid function. Twelve patients had malignancy, including 6 patients with breast cancer, 1 with thyroid follicular cancer, 2 with colon cancer, 1 with ovarian cancer, 1 with melanoma, and 1 with diffuse large B-cell lymphoma. Malignancy was also seen in both seropositive and seronegative patients' pSS and was not statistically significant. No correlation was found between autoantibody positivity and malignancy. However, ANA, anti-SSA, and anti-SSB antibodies were positive in the patient with lymphoma.

Hydroxychloroquine could not be administered because of contraindications in 8 patients and side effects in 7 patients, but it was administered to all patients except these. There were no significant differences in the frequency of corticosteroid, methotrexate, hydroxychloroquine sulfate, or azathioprine treatment in the three groups, although the use of pilocarpine hydrochloride was significantly higher in the *seropositive* group.

### *3.3. Primary Sjögren Syndrome and Cutaneous Vasculitis*

Vasculitis was present in 20 patients. Of these, 12 had a previous diagnosis of pSS, and 8 were diagnosed with pSS during the investigation of vasculitis etiology. The average time to diagnosis for patients who were previously diagnosed was 26 months. Cutaneous lesions included palpable purpura in 18 (90%) patients, erythematous papules in 2 (10%), erythematous macules in 4 (20%), urticarial lesions in 2 (10%), and 3 (15%) ulcers/ischemia. Cutaneous lesions were located in the lower limbs in 20 (100%), the upper limbs in 5 (25%), the trunk in 3 (15%), and general in 2 (10%). After excluding other causes of vasculitis (such as large vessel, medium vessel, and small vessel vasculitis), it was accepted as Sjögren syndrome-associated small vessel vasculitis (SS-SVV) from the Vasculitis associated with systemic disease group. Of these, 15 were leukocytoclastic vasculitis(LCV), 3 were cryoglobulinaemic vasculitis(CV) and 2 were urticarial vasculitis. There were 10 quadruple seropositivity pSS patients, 5 double seropositive, 2 isolated RF positive, and 3 quadruple seronegativity patients with vasculitis (Table 5). Complement was low in all patients with CV and urticarial vasculitis, but it was low in only 2 patients with LCV. Low complement levels were more frequent in patients with vasculitis than in those without vasculitis (7/20 (35%) versus 13/382 (7.1%), $p<0.0001$). All patients were women and the mean age at the time of diagnosis of vasculitis was 45 years, younger than those without vasculitis ( 45 vs. 54.7 years, $p<0.05$). Patients with cutaneous vasculitis had a higher prevalence of ANA positive (75% vs. 70.4%, $p = 0.012$), RF (65% vs. 22.5%, $p<0.0001$), anti-Ro/SS-A antibodies (70% vs. 62.3%, $p = 0.014$), anti-La/SS-B antibodies (55% vs. 16.%, $p<0.000$.) compared with pSS patients without vasculitis. Five patients had arthritis, 1 patient had pleural effusion and diffuse ground-glass areas in the parenchyma, and there was no systemic involvement in all other patients. Three patients with CV had ulcers. These were quadruple seropositive and HCV-negative patients. All patients with LCV received oral corticosteroids and hydroxychloroquine. There were 2 patients with corticosteroid doses >30 mg/day, while others went into remission with lower doses of treatment. Eight patients received immunosuppressive agents (1 cyclophosphamide and 7 azathioprine). Two patients with urticarial vasculitis improved with azathioprine. Oral corticosteroids and hydroxychloroquine were given to 3 patients with CV, cyclophosphamide and then azathioprine to 1 patient, azathioprine to 1 patient, and methotrexate to 1 patient with predominant arthritis. The average follow-up period of patients with CV is only 14 months and they are periodically examined for lymphoma. Fourteen patients had a single episode of cutaneous vasculitis; the remaining 6 suffered relapsing vasculitis. All our vasculitis patients are alive.

**Table 1.** Baseline characteristics of 402 patients with primary Sjögren's syndrome.

| ***Demographic features*** | **Patients (%)** |
|---|---|
| Age, median(range), years | 54.3 (18-84) |
| Gender (female/male) | 378/24 |
| Age of diagnosis (mean±SD) | 48.3 ± 14.8 |
| Disease duration, median (range), months | 18 (1-54) |
| ***Clinical features*** | |

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| | |
|---|---|
| Xerostomia | 372 (92.5%) |
| Xerophtalmia | 360 (89.5%) |
| Schirmer | |
| ≤5 mm | 240(59.7%) |
| 5-10 mm | 100 (24.8%) |
| ≥10 mm | 62 (15.4%) |
| Focus score≥1 | 220 (94.8%) |
| Salivary gland enlargement | 52 (12.9%) |
| Arthralgias | 244 (60.6%) |
| Arthritis | 64 (15.9%) |
| Raynaud' s phenomenon | 16 (3.9%) |
| Lymphadenopathy | 36 (8.9%) |
| Vasculitis-purpura | 20 (4.9%) |
| Interstitial lung disease | 56 (13.9%) |
| NSIP | 50/56 (89.2%) |
| OP | 1/56 (1.7%) |
| UIP | 5/56 (8.9%) |
| Neurological involvement | 20 (4.9%) |
| Central nervous system involvement | 12/20 (60%) |
| Peripheral nervous involvement | 8/20 (40%) |
| Autoimmune thyroiditis | 86(21.3%) |
| Primary biliary cholangitis | 10(2.4%) |
| Renal interstitial disease | 2(0.4%) |
| Malignancy | 12 (2.9%) |
| ***Laboratory features*** | |
| ANA positivity | 284 (70.6%) |
| RF positivity | 96 (23.8%) |
| SSA positivity | 252 (62.6%) |
| SSB positivity | 75 (18.6%) |
| *Quadruple seropositive* | 72 (17.9%) |
| *Quadruple seronegative* | 85 (21.4%) |
| ANA and SSA positivity | 174 (43.2%) |
| ESR (mm/h) (mean±SD) | 35.6± 20.7 |
| CRP (mg/L) (mean±SD) | 12.3 ± 3.2 |
| Anemia | 76 (18.9%) |
| Leukopenia | 42 (10.4%) |
| Lymphopenia | 56 (13.9%) |
| Thrombocytopenia | 22(5.4%) |
| Cryoglobulinaemia | 3 (0.7%) |
| Hypocomplementemia | 20 (4.9%) |
| Hypergammaglobulinemia | 48 (11.9%) |

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| | |
|---|---|
| ***Treatments*** | |
| Corticosteroids | 106 (26.3%) |
| Hydroxychloroquine | 387 (96.2%) |
| Methotrexate | 28 (6.9%) |
| Azathioprine | 18 (4.4%) |
| Cyclophosphamide | 4 (0.9%) |
| Pilocarpine | 42 (10.4%) |

NSIP: Non-specific interstitial pneumonia, OP: Organizing Pneumonia, UIP: Usual interstitial pneumonia.

**Table 2.** Comparison of patients with quadruple seropositivity and quadruple seronegativity.

| **Feature/Clinical manifestation** | ***Quadruple seropositivite*** **n: 72, %** | ***Quadruple seronegativite*** **n: 85, %** | ***p* valvue** |
|---|---|---|---|
| Age, median(range), years | 47.9(18-72) | 59.2 (20-82) | 0.031 |
| Female | 70 (97.2%) | 78 (91.7%) | 0.125 |
| Age of diagnosis(mean ± SD) | 42.4 ± 10.8 | 50.3 ± 13.2 | 0.112 |
| Xerostomia | 70 (97.2%) | 80 (94.1%) | 0.328 |
| Xerophtalmia | 68 (94.0%) | 75 (88.2%) | 0.142 |
| Salivary gland enlargement | 20 (27.7%) | 3(3.5%) | **<0.0001** |
| Arthralgias | 60 (83.3%) | 22 (25.8%) | **<0.0001** |
| Arthritis | 32 (44.4%) | 2 (2.3%) | **<0.0001** |
| Raynaud' s phenomenon | 11 (15.2%) | 0 | **<0.0001** |
| Lymphadenopathy | 21(29.1%) | 2(2.3%) | **<0.0001** |
| Interstitial lung disease | 28(38.8%) | 1(1.1%) | **<0.0001** |
| Vasculitis | 10(13.8%) | 3(3.5%) | **<0.0001** |
| Neurological involvement | 14(19.4%) | 1(1.1%) | **<0.0001** |
| Autoimmune thyroiditis | 42(58.3%) | 8(9.4%) | **<0.0001** |
| Primary biliary cholangitis | 6(8.3%) | 1(1.1%) | 0.023 |
| Renal interstitial disease | 2(2.7%) | 0 | 0.587 |
| Malignancy | 5(6.9%) | 2(2.3%) | 0.016 |
| Cryoglobulinaemia | 3(4.1%) | 0 | 0.348 |
| ESR (mm/h) (mean±SD) | 43.1 ± 14.3 | 32.6± 16.8 | 0.125 |
| CRP (mg/L) (mean±SD) | 10.2 ± 4.4 | 6.3 ± 8.7 | 0.362 |
| Anemia | 32(44.4%) | 10(11.7%) | **<0.0001** |
| Leukopenia | 20 (27.7%) | 4 (4.7%) | **<0.0001** |
| Lymphopenia | 24 (33.3%) | 12(14.1%) | 0.001 |
| Thrombocytopenia | 10(13.8%) | 2(2.3%) | 0.001 |
| Hypergammaglobulinemia | 34 (47.2%) | 4 (4.7%) | **<0.0001** |
| Hypocomplementemia | 5 (6.9%) | 8(9.4%) | 0.352 |

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**Table 3.** Comparison of quadruple seropositive and double seropositive patients.

| Feature/Clinical manifestation | *Quadruple seropositive* n: 72, % | Double seropositive n: 174, % | *p* valvue |
|---|---|---|---|
| Age, median(range), years | 47.9(18-72) | 54.2 (24-84) | 0.091 |
| Female | 70 (97.2%) | 164 (94.2%) | 0.426 |
| Age of diagnosis(mean ± SD) | 42.4 ± 10.8 | 52.3 ± 14.2 | 0.021 |
| Xerostomia | 70 (97.2%) | 161 (92.5%) | 0.242 |
| Xerophtalmia | 68 (94.0%) | 158 (90.8%) | 0.361 |
| Salivary gland enlargement | 20 (27.7%) | 22 (12.6%) | 0.113 |
| Arthralgias | 60 (83.3%) | 138 (79.3%) | 0.516 |
| Arthritis | 32 (44.4%) | 16 (9.1%) | **<0.0001** |
| Raynaud' s phenomenon | 11 (15.2%) | 5 (2.8%) | **<0.0001** |
| Lymphadenopathy | 21(29.1%) | 12 (6.8%) | **<0.0001** |
| Interstitial lung disease | 28(38.8%) | 22 (12.6%) | **<0.0001** |
| Vasculitis | 10(13.8%) | 5 (2.8%) | **<0.0001** |
| Neurological involvement | 14(19.4%) | 5 (2.8%) | **<0.0001** |
| Autoimmune thyroiditis | 42(58.3%) | 30 (17.2%) | **<0.0001** |
| Primary biliary cholangitis | 6(8.3%) | 4 (2.2%) | 0.001 |
| Renal interstitial disease | 2(2.7%) | 0 | 0.657 |
| Malignancy | 5(6.9%) | 3 (1.7%) | 0.325 |
| Cryoglobulinaemia | 3(4.1%) | 0 | 0.388 |
| ESR (mm/h) (mean±SD) | 43.1 ± 14.3 | 36.4± 10.8 | 0.125 |
| CRP (mg/L) (mean±SD) | 10.2 ± 4.4 | 7.6 ± 5.7 | 0.362 |
| Anemia | 32(44.4%) | 24(13.7%) | **<0.0001** |
| Leukopenia | 20 (27.7%) | 15 (8.6%) | **<0.0001** |
| Lymphopenia | 24 (33.3%) | 20(11.4%) | **<0.0001** |
| Thrombocytopenia | 10(13.8%) | 8(4.5%) | **<0.0001** |
| Hypergammaglobulinemia | 34 (47.2%) | 14(8.0%) | **<0.0001** |
| Hypocomplementemia | 5 (6.9%) | 7(4.0%) | 0.456 |

**Table 4.** Comparison of double seropositive patients with quadruple seronegative.

| Feature/Clinical manifestation | Double seropositive n: 174, % | *Quadruple seronegative* n: 85, % | *p* valvue |
|---|---|---|---|
| Age, median(range), years | 54.2 (24-84) | 59.2 (20-82) | 0.162 |
| Female | 164 (94.2%) | 78 (91.7%) | 0.386 |
| Age of diagnosis(mean ± SD) | 52.3 ± 14.2 | 50.3 ± 13.2 | 0.461 |
| Xerostomia | 161 (92.5%) | 80 (94.1%) | 0.345 |

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| | | | |
|---|---|---|---|
| Xerophtalmia | 158 (90.8%) | 75 (88.2%) | 0.526 |
| Salivary gland enlargement | 22 (12.6%) | 3(3.5%) | **<0.0001** |
| Arthralgias | 138 (79.3%) | 22 (25.8%) | **<0.0001** |
| Arthritis | 16 (9.1%) | 2 (2.3%) | **<0.0001** |
| Raynaud' s phenomenon | 5 (2.8%) | 0 | 0.623 |
| Lymphadenopathy | 12 (6.8%) | 2(2.3%) | **<0.0001** |
| Interstitial lung disease | 22 (12.6%) | 1(1.1%) | **<0.0001** |
| Vasculitis | 5 (2.8%) | 3 (3.5%) | 0.568 |
| Neurological involvement | 5 (2.8%) | 1(1.1%) | 0.084 |
| Autoimmune thyroiditis | 30 (17.2%) | 8(9.4%) | 0.032 |
| Primary biliary cholangitis | 4 (2.2%) | 1(1.1%) | 0.021 |
| Renal interstitial disease | 0 | 0 | null |
| Malignancy | 3 (1.7%) | 2(2.3%) | 0.612 |
| Cryoglobulinaemia | 0 | 0 | null |
| ESR (mm/h) (mean±SD) | 36.4± 10.8 | 32.6± 16.8 | 0.421 |
| CRP (mg/L) (mean±SD) | 7.6 ± 5.7 | 6.3 ± 8.7 | 0.512 |
| Anemia | 24 (13.7%) | 10(11.7%) | 0.536 |
| Leukopenia | 15 (8.6%) | 4 (4.7%) | 0.004 |
| Lymphopenia | 20 (11.4%) | 12(14.1%) | 0.426 |
| Thrombocytopenia | 8 (4.5%) | 2(2.3%) | 0.002 |
| Hypergammaglobulinemia | 14 (8.0%) | 4 (4.7%) | 0.004 |
| Hypocomplementemia | 7(4.0%) | 8(9.4%) | 0.001 |

**Table 5.** Serological and histological features of cutaneous vasculitis in 20 patients with pSS.

| Pat. no | ANA | Anti-SSA | Anti -SSB | RF | ComplementC3 low | Complement C4 low | Cryo-globulinaemia | Histologic patern |
|---|---|---|---|---|---|---|---|---|
| 1 | + | + | + | + | + | + | + | CV |
| 2 | + | + | + | + | + | + | + | CV |
| 3 | + | + | + | + | + | + | + | CV |
| 4 | + | + | + | + | - | - | - | LCV |
| 5 | + | + | + | + | - | - | - | LCV |
| 6 | + | + | + | + | + | + | - | urticarial |
| 7 | + | + | + | + | - | - | - | LCV |
| 8 | + | + | + | + | - | - | - | LCV |
| 9 | + | + | + | + | - | - | - | LCV |
| 10 | + | + | + | + | - | - | - | LCV |
| 11 | + | - | + | + | - | - | - | LCV |
| 12 | + | + | - | - | - | - | - | LCV |
| 13 | + | + | - | - | - | - | - | LCV |
| 14 | + | + | - | - | + | + | - | urticarial |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15 | + | + | - | - | - | - | - | LCV |
| 16 | - | - | - | + | - | - | - | LCV |
| 17 | - | - | - | + | - | - | - | LCV |
| 18 | - | - | - | - | - | + | - | LCV |
| 19 | - | - | - | - | - | - | - | LCV |
| 20 | - | - | - | - | - | + | - | LCV |

CV: Cryoglobulinaemic vasculitis, LCV: Leukocytoclastic vasculitis.

**4. Discussion**

The clinical presentation of patients with pSS is highly heterogeneous. Patients may present with varying clinical manifestations ranging from sicca symptoms to systemic disease and lymphoma [2]. Anti-SSA and anti-SSB are the most commonly used immunologic biomarkers in the diagnosis of pSS, but a proportion of patients with pSS are negative for both autoantibodies and patients have varying levels of RF and ANA antibodies. These autoantibodies are known to cause epithelial cell damage, leading to symptoms [5]. A link between pathological Salivary gland ultrasound(SGU) findings and positive autoimmunity has been established, and studies have shown that SGU is a strong predictor of positive SGU findings in patients with sicca symptoms [19]. In another similar study conducted according to the number of autoantibodies; all autoantibodies(quadruple seropositive) were more frequent in patients with pathological SGU findings. The quadruple seropositive group had the highest frequency of pathological SGU findings (78.1%). Most patients in the partial seropositive (have at least 1 of the four autoantibodies) group (60.7%) and all seronegative patients had normal SGU findings [20]. These studies give us information in terms of antibody-associated organ damage. Our pres-ent study investigated the clinical heterogeneity of Turkish pSS patients and found that patients with quadruple, and double antibodies were significantly different from seronegative pSS patients in several aspects. The diagnosis age of quadruple seropositive pSS is significantly younger than patients with double seropositive and quadruple seronegative. The interval between the onset of sicca symptoms and diagnosis was the shortest in quadruple seropositive and averaged 23 months, while it was the longest in quadruple seronegative and was 60 months ($p<0.0001$). This shows us that the presence of autoantibodies in pSS patients helps the early diagnosis. In addition, it shows that those with antibody positivity are diagnosed earlier due to higher disease activity and higher systemic involvement. We found that patients who were positive for autoantibodies were referred earlier to specialists in autoimmune diseases and were diagnosed earlier. The first interesting finding of our study is the prevalence of patients with quadruple seropositive reach-ing almost 17.9 % and quadruple seronegative 21.4% of the total SS population.

Comparing patients with quadruple seropositive and double seropositive pSS, the double positive group was older, clinical findings such as sicca symptoms and arthralgia were more predominant, but systemic involvement such as salivary gland enlargement, arthritis, Raynaud's phenomenon, lymphadenopathy, vasculitis, interstitial lung disease, neurological involvement, autoimmune thyroiditis, primary biliary cholangitis, cytopenias and hypergammaglobulinaemia were less common. Here, we think that the high disease activity and systemic organ involvement are related to anti-SSB/La. It has been observed in previous studies that anti-SSB/La antibody triggers immunoreactivity, increase systemic antibody production, and causes tissue damage by causing inflammatory reaction [21–23]. Arthritis and arthralgia were more common in the quadruple seropositive group than in the others, and we believe that RF positivity was a contributory factor. In a study supporting this, the frequency of articular manifestations was higher in RF-positive pSS patients than in RF-negative patients [4].

In general, the presence of autoan-tibodies correlates with a younger age of onset, female predominance, increased risk of organ involvement, and the presence of other antibodies [24]. In our study, comparing patients with quadruple seropositive and quadruple seronegative pSS, the seronegative group was older, and clinical findings and systemic involvements significantly were less common(Table 2). This finding is important because it shows that seronegative patients with seropositive antibodies are more likely to have severe disease. The autoantibody status represents a

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main factor driving the phenotypic expression of pSS and may help in the identification of subgroups of pa-tients with poorer prognosis [3].

Cutaneous vasculitis is observed in approximately 4 to 10 percent of patients with pSS [25–28]. One possible approach to classifying SS-associated small vessel vasculitis (SVV) may be to distinguish between cryoglobulinemic vasculitis, urticarial vasculitis, and non-cryoglobulinemic, non-urticarial leukocytoclastic vasculitis [14]. In the same study; CV was diagnosed in 14 (27%) of the 52 SS patients with cutaneous vasculitis [14]. The prevalence of cryoglobulins in pSS is 9–15%, the most common type being type III mixed cryoglobulinemia [29]. In our study, cryoglobulinemia was positive in 3 patients (0.7%). Three patients had cutaneous vasculitis with hypocomplementemia and were in the quadruple seropositive group. In our study, there was less cryoglobulinemia compared to other studies. The reason for this is that we only studied vasculitis patients, neurological involvement, and RF positivity together with hypocomplementemia. We observed that the lesions were more generalized and accompanied by necrosis in CV compared to other patients with vasculitis. In the same study; LCV was diagnosed in the remaining 26 (50%) patients and urticarial vasculitis was diagnosed in 11 (21%) of the 52 pSS patients with cutaneous vasculitis [14]. In our study, LCV was seen in 15 (75%) and urticarial vasculitis in 2 (10%). We can associate these differences with the increase in antibody positivity. Because the number of autoantibodies was higher in our study. We observed that the duration of remission in patients with hypocomplementemia was long and relapses were high. In addition, higher corticosteroid doses were used in these patients. With these data, we recommend that more aggressive and effective immunosuppressive therapy be initiated from the beginning in patients with hypocomplementatemia. The development of cutaneous vasculitis may have important prognostic implications, as such patients are more likely to develop other extraglandular manifestations, including lymphoma, and die from disease-related complications than those without vasculitis [14,30,31]. Raynaud's phenomenon, vasculitis, renal interstitial disease, and cryoglobulinemia were never seen in quadruple seronegative patients. These findings suggest that autoantibody-related and circulating immune complexes play a major role in organ damage and may be more frequent in the seropositive group. However, the clinical expression of the disease should not be interpreted only from autoantibodies but the counter immunoregulation and genetic features should be also considered, and therefore not all seropositive patients are finally expected to develop a worse clinical phenotype.

There were several limitations in our study. This study was performed in a single center in Turkey, retrospective design and the sample size included was relatively small, especially the pSS-associated small vessel vasculitis. The relationship between disease activity and systemic involvement could not be evaluated due to a retrospective study. Cryoglobulinemia was studied only in vasculitis patients, with neurological involvement, and only in patients with RF positivity and hypocomplementatemia. Since it was not studied in other patients, we encountered it less frequently than in previous studies. We could not make a histological comparison between the 3 groups because they were evaluated by different pathologists and most of them had focus scores>1 or aggregate number>1. The follow-up period is 18 months and because of this short follow-up period, we could not observe the long-term complications yet. We were not able to do a bone marrow biopsy on some of the patients with cytopenia because they did not give their consent.

## 5. Conclusions

Extraglandular manifestations are more common in quadruple seropositive patients than in double seropositive and quadruple seronegative patients with pSS. We think that antibody positivity is an important factor, especially in circulating immune complexes and antibody-related organ damage such as vasculitis, Raynaud's phenomenon, and cryoglobulinemia. Primary SS patients with cutaneous vasculitis had a higher prevalence of immunological features (ANA, RF, anti-Ro/SS-A, anti-La/SS-B, and cryoglobulins). We observed two risk factors in patients with vasculitis who received high-dose corticosteroids for treatment, were resistant to treatment, and had common relapses. These were hypocomplementemia and cryoglobulinemia. The presence of cutaneous vasculitis, hypocomplementaemia, and cryoglobulinemia may have important prognostic implications in patients with primary SS.

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**Informed Consent Statement:** Written informed consent was obtained from the patients.

**Data Availability Statement:** The data presented in this study are available upon reasonable request from the corresponding author.

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