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Elevated Serum Levels of Soluble Transferrin Receptor Are Associated with an Increased Risk of Cardiovascular, Pulmonary, and Hematological Manifestations and a Decreased Risk of Neuropsychiatric Manifestations in Systemic Lupus Erythematosus Patient

Agnieszka Winikajtis-Burzyńska , Marek Brzosko , Hanna Przepiera-Będzak *

Posted Date: 6 November 2023

doi: 10.20944/preprints202311.0317.v1

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Elevated Serum Levels of Soluble Transferrin Receptor Are Associated with an Increased Risk of Cardiovascular, Pulmonary, and Hematological Manifestations and a Decreased Risk of Neuropsychiatric Manifestations in Systemic Lupus Erythematosus Patients

Agnieszka Winikajtis-Burzyńska [1], Marek Brzosko [2] and Hanna Przepiera-Będzak [2,*]

[1] Individual Laboratory for Rheumatologic Diagnostics, Pomeranian Medical University in Szczecin, Unii Lubelskiej 1, 71-252 Szczecin, Poland
[2] Department of Rheumatology, Internal Medicine, Geriatrics and Clinical Immunology, Pomeranian Medical University in Szczecin, Unii Lubelskiej 1, 71-252 Szczecin, Poland
* Correspondence: hanna.przepiera.bedzak@pum.edu.pl; Tel.: +48914253321; Fax: +48914253344

Abstract: The aim of this study was to analyze the relationship between the serum levels of soluble transferrin receptor (sTfR) and interleukin 4 (IL-4), and disease activity and organ manifestations in SLE patients. We studied 200 SLE patients and 50 controls. We analyzed disease activity, organ involvement, serum sTfR, IL-4, and interleukin-6 (IL-6) levels, and antinuclear and antiphospholipid antibody profiles. The median serum levels of sTfR ($p>0.000001$) and IL-4 ($p<0.00001$) were higher in the study group than in the controls. SLE patients, compared to the controls, had significantly lower HGB levels ($p<0.0001$), a lower iron concentration ($p=0.008$), a lower value of total iron-binding capacity (TIBC) ($p=0.03$), and lower counts of RBC ($p=0.004$), HCT ($p=0.0004$), PLT ($p=0.04$), neutrophil ($p=0.04$), and lymphocyte ($p<0.0001$). Serum sTfR levels were negatively correlated with lymphocyte ($p=0.0005$), HGB ($p=0.0001$) and HCT ($p=0.008$), and positively correlated with IL-4 ($p=0.01$). Elevated serum sTfR >2.14 mg/dL was associated with an increased risk of myocardial infarction (OR: 10.6 95 CI 2.71–464.78; $p=0.001$), ischemic heart disease (OR: 3.25 95 CI 1.02–10.40; $p=0.04$), lung manifestations (OR: 4.48 95 CI 1.44–13.94; $p=0.01$), and hematological manifestations (OR: 2.07 95 CI 1.13–3.79; $p=0.01$), and with a reduced risk of neuropsychiatric manifestations (OR: 0.42 95 CI 0.22–0.80; $p=0.008$). Serum IL-4 was negatively correlated with CRP ($p=0.003$), and elevated serum IL-4 levels > 0.17 mg/l were associated with a reduced risk of mucocutaneous manifestations (OR: 0.48 95 CI 0.26–0.90; $p=0.02$). In SLE patients, elevated serum levels of sTfR were associated with an increased risk of cardiovascular, pulmonary, and hematological manifestations, and with a decreased risk of neuropsychiatric manifestations. In contrast, elevated serum IL-4 levels were associated with a decreased risk of mucocutaneous manifestations.

Keywords: systemic lupus erythematosus; serum soluble transferrin receptor; interleukin 4; organ manifestations

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease. The clinical picture of the disease varies from a mild course to life-threatening disease [1]. In the course of the disease, skin, mucosal, hematological, musculoskeletal, cardiovascular, neurological, gastrointestinal, pulmonary and renal organ manifestations may develop [1,2].

Hematologic disorders associated with abnormal iron metabolism are common in SLE. A lack of normal regulation in iron homeostasis can cause anemia of chronic disease (ACD) or iron deficiency anemia (IDA) [3]. Anemia in the course of SLE occurs in about 50% of patients [3,4]. The incidence of anemia is influenced by many factors, such as inflammation, renal failure, gastrointestinal complications and hemolysis. Numerous studies in SLE patients have reported that

 doi:10.20944/preprints202311.0317.v1

the prevalence of ACD ranges from 30% to 80% [4,5]. In many SLE patients, the differentiation of ACD and IDA is difficult. The determination of soluble transferrin receptor (sTfR), which is the plasma-soluble form of the transferrin receptor and is an indicator of tissue iron deficiency, is helpful in differentiating between ACD and IDA. Elevated sTfR concentrations are indicative of an existing iron body deficiency or IDA. In ACD, sTfR concentrations are unchanged [6].

Interleukin 4 (IL-4) is an anti-inflammatory cytokine with a broad spectrum of effects. IL-4 stimulates the proliferation and differentiation of B lymphocytes and Th2 lymphocytes, inactivates the differentiation of Th1 lymphocytes and regulatory T cells (Treg), affects the production of IgE and IgG4, and is involved in granulopoiesis and erythropoiesis [7,8]. The role of IL-4 in SLE patients is ambiguous [8,9]. The available literature lacks a comprehensive analysis of the serum concentrations among sTfR and IL-4 and iron metabolism parameters, as well as organ manifestations in SLE patients.

The aim of this study was to analyze the relationship among serum sTfR and IL-4 levels, disease activity and organ manifestations in SLE patients.

2. Results

The characteristics of the study group are shown in Table 1.

Table 1. Clinical characteristics of systemic lupus erythematosus patients and healthy controls.

Assessed Parameters		**Study group n = 200 Mean ± SD Number (%)**	**Control group n = 50 Mean ± SD Number (%)**	**p**
Sex		F: 181 (90.5); M: 19 (9.5)	F: 44 (88.0); M: 6 (12.0)	0.6
Age (years)		46.97 ± 13.73	-	-
Disease duration (years)		10.40 ± 9.10	-	-
SLEDAI		10.07 (5.81)	-	-
Constitutional		52 (26.50)	-	-
Mucocutaneous			-	-
	Any change	135 (68.90)		
	Malar rash	115 (57,50)		
	Discoid rash	12 (6,00)		
	Oral ulcerations	44 (22,00)		
Arthritis		155 (77,50)	-	-
Heart		80 (40.4)	-	-
Myocardial infarction		10 (5.0)		
Ischemic heart disease		17 (9.6)	-	-
Hypertension		65 (32.5)	-	-
Lung			-	-
	Any change	12 (10.0)		
	Interstitial changes	6 (3.0)		
	Nodular lesions	4 (2.0)		
	Pleural effusion	2 (1.0)		
Haematologic involement				
	Any change	139 (69.50)	-	-
	Hemolytic anaemia	10 (8,50)		
	Deficiency anaemia	82 (43,40)		
	Leucopenia	75 (37,69)		
	Lymphopenia	87 (43,50)		
	Trombocytopenia	42 (21,11)		
Vascular system		29 (14.8)	-	-

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Neuropsychiatric	68 (34.34)	-	-
Renal lupus	43 (21.50)	-	-
Treatment			
Antimalarials	154 (77)	-	-
Cs	162 (81)	-	-
Azathioprine	30 (15)	-	-
Cyclophosphamide	43 (21.5)	-	-
MMF	10 (5)	-	-
Methotrexate	7 (3.5)	-	-
Cyclosporin A	4 (2)	-	-
Immunoglobulins	12 (6)	-	-
Epratuzumab	2 (1)	-	-

n: number; F: female; M: men; SLEDAI: Systemic Lupus Erythematosus Activity Index; Cs: corticosteroids, MMF: mycophenolate mofetil.

The median concentration of sTfR was higher in the study group than in the control group (p>0.000001). The median serum IL-4 concentration was higher in the study group than in the control group (p<0.00001) (Table 2).

Analysis of the hematological parameters showed that SLE patients, compared to the control group, had a significantly lower count of red blood cells (RBC) (p=0.004), hematocrit (HCT) (p=0.0004), platelets (PLT) (p=0.04), neutrophil (p=0.04) and lymphocytes (p<0.0001), a lower HGB concentration (p<0.0001), and significantly lower values of indices: mean corpuscular hemoglobin (MCH) (p=0.03) and mean corpuscular hemoglobin concentration (MCHC) (p<0.0001). There was no significant difference between the study group and the control group regarding white blood cell (WBC) count, the mean corpuscular volume (MCV) index value and the number of reticulocytes (Ret) (all p>0.05) (Table 2).

Analysis of the iron metabolism parameters showed that SLE patients, compared to the control group, had significantly lower iron (Fe) concentrations (p=0.008) and lower total iron-binding capacity (TIBC) values (p=0.03). There was no significant difference regarding the unsaturated iron-binding capacity (UIBC) value, and the ferritin, transferrin (Tf), and transferrin saturation (TfS) concentrations between the study and the control group (all p>0.05) (Table 2).

Table 2. Laboratory characteristics of systemic lupus erythematosus patients and healthy controls.

Assessed Parameters	**Study group n = 200 Mean ± SD Median (Q1, Q3) Number (%)**	**Control group n = 50 Mean ± SD Median (Q1, Q3) Number (%)**	**p**
Sex	F: 181 (90.5); M: 19 (9.5)	F: 44 (88.0); M: 6 (12.0)	0.6
IL-4 (pg/ml)	0.00 (0.00, 1.58)	0.00 (0.00, 0.00)	<0.00001
sTfR [mg/l]	2.15 (1.6, 2.83)	1.51 (1.22, 2.04)	<0.00001
IL-6 (pg/ml)	2.50 (0.89, 5.40)	0.84 (0.30, 1.26)	<0.00001
ESR (mm/h)	16.00 (8.00, 30.00)	6.00 (4.00, 10.00)	< 0.0001
CRP (mg/l)	1.89 (1.00, 5.83)	-	-
Complement factor C3 (mg/dl)	97.45 ± 25.2	-	-
Complement factor C4 (mg/dl)	16.86 ± 7.52	-	-
Fibrinogen (mg/dl)	349.5 ± 108.4	280.0 ± 65.5	0.0001
Positive direct Coombs' test	25 (27.17)	-	-
False positive syphilis test (VDRL)	2 (1.90)	-	-
Hematological parameters			
WBC (103/μl)	5.71 (4.36, 7.46)	5.97 (4.73, 6.65)	0.7
Lymphocytes (103/μl)	1.36 (0.99, 1.82)	1.81 (1.57, 2.17)	< 0.0001

 doi:10.20944/preprints202311.0317.v1

Neutrofils (103/µl)	3.65 (2.55, 5.31)	3.33 ± 1.33	0.04
HGB (g/dl)	12.67 ± 1.70	13.85 ± 1.08	< 0.0001
RBC (mln/µl)	4.39 ± 0.53	4.65 ± 0.40	0.004
HGB (g/dl)	12.67 ± 1.70	13.85 ± 1.08	<0.0001
HCT (%)	38.05 ± 4.51	40.49 ± 3.03	0.0004
MCV (fl)	86.93 ± 6.55	87.32 ± 4.24	0.7
MCH (pg)	28.97 ± 2.74	29.87 ± 1.67	0.03
MCHC (g/dl)	33.26 ± 1.32	34.21 ± 0.89	<0.0001
PLT (103/µl)	227.4 ± 78.2	251.2 ± 55.6	0.04
Ret (‰)	10.00 (7.00, 14.00)	10.99 ± 5.07	0.5
Iron metabolism parameters			
Ferritin (ng/ml)	55.3 (23.6, 133.6)	43.1 (20.4, 109.6)	0.2
Tf (mg/dl)	260.0 ± 53.3	275.8 ± 54.6	0.06
Fe ug/dl	81.5 ± 46.2	100.7 ± 43.4	0.008
TIBC (ug/dl)	309.8 ± 64.6	331.0 ± 51.4	0.03
UIBC ug/dl	227.4 ± 84.0	230,3 ± 67,6	0.8
TfS (%)	27.40 ± 16.45	31,15 ± 13,01	0.14
Immunological assessment			
ANA IgG	198 (99.00)	1 (2)	-
Anti-dsDNA IgG	86 (45.70)	-	-
Anti-NuA IgG	57 (32.90)	-	-
Anti-Sm IgG	9 (5.10)	-	-
Anti-SS-A/Ro IgG	68 (39.10)	-	-
Anti-SS-B/La IgG	25(14.90)	-	-
Anti-SS-A/Ro IgG	68 (39.10)	-	-
Anti-SS-B/La IgG	25(14.90)	-	-
Anti-ARPA IgG	6 (3.50)	-	-
Anti-histones IgG	24(14.00)	-	-
Anti-U1-snRNP IgG	22 (12.60)	-	-
Anti-CL IgG	49 (28.00)	-	-
Anti-CL IgM	66 (37.70)	-	-
Anti-ß2-GPI screen IgA, IgG, IgM	61 (35.30)	-	-

IL-4: interleukin 4; IL-6: interleukin 6; sTfR: soluble transferrin receptor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; WBC: white blood cell; HGB: hemoglobin; RBC: red blood cells; PLT: blood platelets; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelets; Ret: reticulocytes; Tf: transferrin; Fe: iron; TIBC: total iron-binding capacity; UIBC: unsaturated iron-binding capacity; TfS: transferrin saturation; VDRL: Venereal Diseases Research Laboratory; ANA :anti-nuclear antibodies; Anti-dsDNA: anti-double stranded DNA antibodies; Anti-NuA: anti-nucleosome antibodies; Anti-Sm: anti-Smith antibodies; Anti-SS-A/Ro: anti-Rose antibodies; Anti: ARPA: anti-ribosomal P protein antibodies; Anti-CL: anticardiolipin antibodies; Anti-B2GP-I: β2-glycoprotein I antibodies; Ig A: immunoglobulin A; Ig G: immunoglobulin G; Ig M: immunoglobulin M.

SLE patients showed a positive correlation between serum sTfR and IL-4 levels (p=0.01). There was no significant correlation between sTfR levels and patients' age, disease duration and IL-6 levels (all p>0.05).

In SLE patients, a negative correlation was found between serum sTfR levels and HGB levels (p=0.0001), HCT (p=0.008), MCV (p=0.0001), MCH (p<0.00001), and MCHC indexes (p<0.00001), and the lymphocyte count (p=0.0005). A positive correlation was found between the sTfR and WBC count (p=0.03), Ret count (p=0.001) and neutrophil count (p=0.002). There was no significant correlation between serum sTfR and RBC, PLT, monocytes and eosinophils levels (all p>0.05) (Table 3).

The study group showed a negative correlation between the serum sTfR and ferritin concentration (p<0.00001), Fe concentration (p<0.00001) and TfS index (p<0.00001). In SLE patients, there was a positive correlation between the serum sTfR and Tf concentration (p=0.001), TIBC

 doi:10.20944/preprints202311.0317.v1

(p=0.007), and UIBC (p<0.00001). There was no significant correlation between serum sTfR levels and CRP levels, fibrinogen levels, ESR values, and folic acid and vitamin B12 levels (all p>0.05) (Table 3).

A multivariate logistic regression analysis and stepwise analysis showed that in SLE patients, elevated serum sTfR >2.14 mg/dL was associated with an increased risk of myocardial infarction (OR: 10.6 95 CI 2.71–464.78; p=0.001), ischemic heart disease (OR: 3.25 95 CI 1.02–10.40; p=0.04), lung involvement (OR: 4.48 95 CI 1.44–13.94; p=0.01), and hematological manifestations (OR: 2.07 95 CI 1.13–3.79; p=0.01), and with a reduced risk of neuropsychiatric manifestations (OR: 0.42 95 CI 0.22–0.80; p=0.008) (Table 3).

In SLE patients, there was no significant correlation between serum IL-4 levels and IL-6 levels (all p>0.05). In the study group, there was no significant correlation between serum IL-4 levels and patients' age, disease duration, and blood count parameters: WBC, neutrophils, lymphocytes, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, and Ret (all p>0.05) (Table 3). There were no significant correlations between serum IL-4 levels and other indicators of iron metabolism (all p>0.05) (Table 3). No significant correlation was found between serum IL-4 levels and vitamin B12, and folic acid levels (all p>0.05) (Table 3). In SLE patients, there was a negative correlation between serum IL-4 and CRP levels (p=0.003) (Table 3). There was no significant correlation between serum IL-4 and fibrinogen levels or ESR values (all p>0.05) (Table 3).

Table 3. Laboratory characteristics of systemic lupus erythematosus patients and healthy controls.

Assessed Parameters	**Levels of sTfR [mg/l]**		**Levels of IL-4 [pg/ml]**	
	Spearman's rank correlation coefficient, R	**p**	**Spearman's rank correlation coefficient, R**	**p**
WBC (tys/µl)	0.15	0.03	-0.11	0.1
RBC (mln/µl)	0.00	1.0	-0.07	0.3
HGB (g/dl)	-0.28	0.0001	-0.04	0.6
HCT (%)	-0.19	0.008	-0.04	0.5
MCV (fl)	-0.28	0.0001	0.01	0.9
MCH (pg)	-0.36	<0.00001	-0.01	0.9
MCHC (g/dl)	-0.40	<0.00001	0.00	1.0
Ret (‰)	0.23	0.001	0.01	0.8
PLT (tys/µl)	0.04	0.6	-0.06	0.4
Neutrofils (103/µl)	0.21	0.002	-0.03	0.6
Lymphocytes (103/µl)	-0.24	0.0005	-0.06	0.4
Monocytes (103/µl)	0.02	0.8	0.04	0.6
Basophils (103/µl)	-0.15	0.04	-0.01	0.9
Eosinophils (103/µl)	-0.07	0.3	0.08	0.3
Ferritin (ng/ml)	-0.29	<0.00001	-0.04	0.5
Tf (mg/dl)	0.24	0.001	-0.09	0.2
TIBC (µg/dl)	0.24	0.0007	-0.09	0.2
Fe (µg/dl)	-0.39	<0.00001	-0.03	0.7
TfS (%)	-0.42	<0.00001	0.00	1.0
UIBC(µg/dl)	0.38	<0.00001	-0.05	0.5
sTfR (nmol/l)			0.17	0.01
Folic acid (ng/ml)	-0.02	0.8	-0.08	0.3
Vitamin B12 (pg/ml)	-0.07	0.3	0.00	1.0
CRP (mg/l)	0.08	0.3	-0.22	0.003
ESR (mm/h)	0,09	0.2	-0.07	0.3
SLEDAI	-0,02	0.7	-0.12	0.1

 doi:10.20944/preprints202311.0317.v1

IL-4: interleukin 4; sTfR: soluble transferrin receptor; WBC: white blood cell; RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; Ret: reticulocytes; PLT: blood platelets; Tf: transferrin; TIBC: total iron-binding capacity; Fe: iron; TfS: transferrin saturation; UIBC: unsaturated iron-binding capacity; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SLEDAI: Systemic Lupus Erythematosus Activity Index.

In a multivariate logistic regression analysis model and stepwise analysis, elevated serum IL-4 and elevated serum sTfR were not associated with the presence of antibodies in SLE patients (all p>0.05) (Table 4).

In a multivariate logistic regression analysis model and stepwise analysis, elevated serum IL-4 levels > 0.17 mg/L in SLE patients were associated with a reduced risk of mucocutaneous manifestations (OR: 0.48 95 CI 0.26–0.90; p=0.02) (Table 4).

Table 4. A logistic regression model of the OR of the increased serum sTfR and IL-4 levels, and organ involvement in patients with systemic lupus erythematosus.

Organ manifestations	Levels sTfR >2.14 mg/l			**Levels of IL-4 > 0.17 pg/ml**		
	OR	95% CI	p	OR	95% CI	P
Constitutional	0.79	0.42 – 1.49	0.4	0.78	0.41 – 1.52	0.4
Mucocutaneous	0.74	0.40 – 1.35	0.3	0.48	0.26 – 0.90	0.02
Arthritis	0.74	0.38 – 1.42	0.3	0.76	0.39 – 1.47	0.4
Heart	1.14	0.65 – 2.02	0.6	1.04	0.58 – 1.86	0.8
Myocardial infarction	10.60	2.71 – 464.78	0.001	0.36	0.07 – 1.74	0.2
Ischemic heart disease	3.25	1.02 – 10.40	0.04	0.29	0.08 – 1.05	0.05
Hypertension	0.93	0.51 – 1.71	0.8	0.59	0.31 – 1.10	0.09
Lung	4.48	1.44 – 13.94	0.01	1.06	0.41 – 2.72	0.9
Haematological	2.07	1.13 – 3.79	0.01	0.96	0.53 – 1.77	0.9
Vascular system	1.47	0.66 – 3.26	0.3	1.34	0.61 – 2.97	0.4
Neuropsychiatric	0.42	0.22 – 0.80	0.008	0.59	0.31 – 1.13	0.1
Renal lupus	1.16	0.59 – 2.29	0.6	1.00	0.50 – 2.00	0.9

OR: Odds ratio, adjusted for gender and age; 95% CI: confidence interval; IL-4: interleukin 4; sTfR: soluble transferrin receptor.

3. Discussion

Systemic lupus erythematosus is an autoimmune disease during the course of which the occurrence of hematological and other organ manifestations is a significant clinical problem, and a variety of cytokines, such as IL-4, IL-6 and interleukin 10 (IL-10), can have a significant impact on that.

We conducted a study of SLE patients in whom serum levels of sTfR and IL-4 were evaluated in association with selected markers of disease activity, namely hematological and other organ manifestations, iron metabolism parameters and antibodies.

SLE patients have hematologic abnormalities, which can either appear as an independent symptom or accompany other clinical manifestations [3]. Tomczyk-Socha M. et al. [10] compared the prevalence of hematologic manifestations in 71 SLE patients with short and long disease duration in a Caucasian population. They found the presence of hematological disorders in 53.5% of SLE patients at the time of diagnosis. In SLE patients with short and long disease duration, they found anemia in 33.8% and 42.3%, respectively, leukopenia in 32.4% and 33.8%, respectively, and thrombocytopenia in 18.3% and 12.7%, respectively. In another study, in SLE patients from Turkey, the presence of hematologic symptoms was found in 67.3% of the subjects, of which AIHA was present in 6.5% and thrombocytopenia in 18.0% of the patients [11]. In SLE patients from Morocco,

 doi:10.20944/preprints202311.0317.v1

Zian Z. et al. [12] found hematologic disorders in 46.0% of patients, including AIHA in 16.0%, lymphopenia in 30.0%, leukopenia in 8.0% and thrombocytopenia in 8.0%. In our study, hematological symptoms were present in 69.5% of SLE patients, including AIHA in 8.5%, anemia of other types (ACD, IDA and ACD with IDA) in 43.4%, lymphopenia in 43.5%, leukopenia in 37.7% and thrombocytopenia in 21.1%. These results are in agreement with data presented by other investigators [10–12].

Soluble transferrin receptor is the plasma-soluble form of the transferrin receptor and is an indicator of tissue iron deficiency. Elevated sTfR concentrations are indicative of an existing iron deficiency or IDA. In ACD, the concentration of sTfR is unchanged [6]. In our study, we found significantly higher serum sTfR concentrations in the study group compared to the control group. Elevated serum sTfR levels in SLE patients may be indicative of impaired iron metabolism, suggesting iron deficiency or increased erythropoiesis. In SLE patients, elevated serum sTfR levels may suggest the presence of IDA [13–15]. In our study, we demonstrated a positive correlation between serum sTfR levels and IL-4 levels in SLE patients. In the available literature, we did not find any studies showing a direct association between sTfR levels and IL-4 levels in SLE patients. In Kuvibidila S.R. et al.'s [16] study, conducted on an animal model, it was found that serum IL-4 was positively correlated with Fe, the HGB concentration and the HCT value. The hemoglobin level is an anemia marker with a low-sensitivity and low-specificity, and is unable to distinguish the type of anemia. HCT is an unreliable indicator in the diagnosis of anemia [17]. Reduced serum Fe levels stimulate sTfR synthesis. Our results suggest that IL-4 may have a stimulatory effect on the development of iron deficiency anemia in SLE patients.

IL-4 is a monomeric glycoprotein produced by Th2 lymphocytes, NK cells, mast cells and basophils [18,19]. Interleukin 4 is an anti-inflammatory cytokine that inhibits the production of pro-inflammatory cytokines and acute-phase proteins such as haptoglobin, CRP, and albumin [20]. Observations of the results of IL-4 concentrations in SLE patients obtained by different investigators are divergent [8,21,22]. Zhou H et al. [21] obtained comparable results regarding serum IL-4 concentrations in SLE patients and controls. On the other hand, Guimarães P.M. et al. [20] showed significantly reduced serum IL-4 concentrations in SLE patients compared to healthy subjects. In contrast, other researchers showed that IL-4 serum levels in SLE patients were significantly higher compared to the controls, which is in agreement with our results [10].

Arora V. et al. [22] found a negative correlation between serum IL-4 levels and disease activity, as measured using the SLEDAI scale, and linked this to the anti-inflammatory and immunosuppressive effects of IL-4. In our study, we found no significant correlation between serum IL-4 levels and patient age, duration and disease activity, as measured using the SLEDAI scale, but we demonstrated a negative correlation between serum IL-4 and CRP levels, confirming the anti-inflammatory effect of this cytokine in SLE patients.

In a study by Zhou H. et al. [21], it was shown that patients with positive anti-double-stranded DNA antibodies (anti-dsDNA) had lower IL-4 levels compared to patients with negative results regarding anti-dsDNA antibodies. In our study, no such relationship was confirmed.

The determination of blood count and iron metabolism parameters is crucial in diagnosing the type of anemia. Determining the type of anemia in SLE patients using conventional laboratory parameters is often difficult. The analysis of iron metabolism parameters in our study showed that SLE patients had significantly lower Fe and TIBC levels compared to the controls, which is in line with previous findings [4,23]. The results of a study conducted by Kunireddy N. et al. [4] in SLE patients showed significantly lower Fe, TIBC and Tf levels compared to the controls, as well as elevated ferritin and hepcidin levels. In another study of SLE patients, elevated ferritin and reduced Tf and TIBC levels were observed compared to the controls [24]. In our study, there was no significant difference in the UIBC, Tf and ferritin levels, and TfS index between the study group and the control group. However, we showed that reduced ferritin levels correlated with the risk of IDA, which is consistent with previous studies [4].

In the course of SLE, patients may develop lesions in multiple organs, but we do not have markers to predict that.

 doi:10.20944/preprints202311.0317.v1

Iron disturbance is associated with abnormal cardiomyocyte function. Myocardial manifestations are most often accompanied by iron deficiency and/or anemia, which appear to be important factors contributing to patients' deterioration. Recently, sTfR levels were proposed as a potential new marker of iron metabolism in cardiovascular diseases. In the AtheroGene study, increased serum sTfR levels were strongly associated with future myocardial infarction and cardiovascular death [25]. In our study, we showed that elevated serum sTfR levels (>2.14 mg/dL) were associated with an increased risk of cardiovascular manifestations such as myocardial infarction and ischemic heart disease in patients with SLE.

The results of Kalkan G et al.'s [26] study suggested that there is a possible association between the functional IL4 VNTR genetic polymorphism and oral mucosal diseases of Turkish SLE patients. In our study, we showed that elevated serum IL-4 levels (>0.17 pg/ml) are associated with a reduced risk of skin and mucosal lesions in SLE patients. Conducting further studies to identify the cytokines involved in organ manifestation may be helpful in personalized immunotherapy for SLE patients.

4. Materials and Methods

4.1. Patients and Controls

We studied 200 Caucasian patients with confirmed diagnoses of SLE and recorded data concerning their age, sex, disease duration, organ involvement, disease activity, and treatment. The control group consisted of 50 healthy individuals (44 females, 6 males) age- and sex-matched with the study group. The ethics committee of the Pomeranian Medical University in Szczecin approved this study (KB-0012/11/13), and all participants provided informed consent.

The diagnosis of SLE was established according to the American College of Rheumatology (ACR) criteria of 1982 (modified in 1997) and the classifications developed by the Systemic Lupus International Collaborating Clinics (SLICC) of 2012 [27]. The disease activity of SLE was assessed according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scale in a modified version: SLEDAI-2000 (SLEDAI-2K) [28].

4.2. Laboratory and Serological Diagnostics

For the estimation of sTfR, IL-4 and IL-6 levels, serum was stored at -80°C until analysis using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) method using the Human sTfR Immunoassay Quantikine® ELISA kit, Human IL-4 Immunoassay Quantikine® ELISA kit, and the Human IL-6 Immunoassay Quantikine® ELISA kit from R&D Systems, United States.

IgG antinuclear antibodies (ANA) were assessed in a HEp-2 cell line contaminated by CVCL-0030 cervical adenocarcinoma human HeLa using indirect immunofluorescence assay (IIFA). Monospecific tests were also performed using the ELISA method to detect anti-double-stranded DNA (anti-dsDNA), anti-Sm, anti-SS-A/Ro, anti-SS-B/La, anti-nucleosome (anti-NuA), anti-ribosomal P protein, anti-histone, and anti-U1-RNP antibodies (EUROIMMUN AG Medizinische Labordiagnostika tests, Lűbeck, Germany). The reference values of ANA were established as absent when the titer was < 1:160 and present when the titer was ≥ 1:160. The titers were divided into three groups: low titers from 1:160 to 1:320, medium titers from 1:640 to 1:1280, and high titers > 1:1280.

The profiles of anti-phospholipid antibodies (aPL), including anticardiolipin (aCL) and anti-beta 2 glycoprotein I (aβ2-GPI), were determined using the ELISA method (EUROIMMUN AG Medizinische Labordiagnstika tests, Lűbeck, Germany). The lupus anticoagulant (LA) was tested using coagulological methods according to the criteria of the International Society of Thrombosis and Hemostasis [29].

Additionally, blood was taken for the assessment of ESR (Westergren method), C-reactive protein (CRP) (turbidimetric nephelometry), fibrinogen (Clauss method), and complement factors C3 and C4 (nephelometry) levels.

Blood count examination was performed using an automated method with XN-2000 and XN-550 hematology instruments from Sysmex Japan, using fluorescence flow cytometry (FFC).

 doi:10.20944/preprints202311.0317.v1

The following blood morphology parameters were determined: hemoglobin (HGB), hematocrit (HCT), red blood cells (RBC), white blood cells (WBC) and blood platelets (PLT). The following blood cell indices were determined: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

The following parameters of iron metabolism were determined: iron (Fe), ferritin, (Tf), transferrin saturation (TfS), total iron-binding capacity (TIBC), and unsaturated iron-binding capacity (UIBC), using COBAS 8000 from Roche Diagnostics.

The vitamin B12 concentration and folic acid concentration were determined using the ECLIA method with COBAS 8000 from Roche Diagnostics.

4.3. Statistical Analysis

Data distributions were evaluated using the Kolmogorov–Smirnov test. Data are presented as means (SD) and medians (Q1, Q3). The R values of correlations were also determined. The groups were compared using a Student's t-test, Mann–Whitney U test, and Kruskal–Wallis test. The parameters were evaluated using a Pearson's chi-squared test ($\chi 2$), logistic regression analysis, and stepwise analysis, and a $p < 0.05$ was considered statistically significant. All statistical data were analyzed using STATA 11: license number 30110532736 (StatSoft Inc, Tulsa, Oklahoma, United States).

5. Conclusions

In SLE patients, elevated serum levels of sTfR were associated with an increased risk of cardiovascular, hematological, and pulmonary manifestations, and a decreased risk of neuropsychiatric manifestations. In contrast, elevated IL-4 levels were associated with a decreased risk of mucocutaneous lesions.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

Author Contributions: Agnieszka Winikajtis-Burzyńska participated in the design and coordination of the study, carried out the immunoassays, performed the statistical analysis, and drafted the manuscript. Marek Brzosko participated in the design and coordination of the study and helped draft the manuscript. Hanna Przepiera-Będzak participated in the design and coordination of the study and drafted the manuscript. All authors read and approved the final manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics committee of Pomeranian Medical University in Szczecin (KB-0012/11/13).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgments: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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