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Research Article

Lymphopenia and Pediatric Systemic Lupus Erythematosus More than a Diagnostic Factor, an Potential Indicator of Severity

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Abstract

Pediatric systemic lupus erythematosus is a severe autoimmune disease. The diagnostic classifications employed for this ailment incorporate lymphopenia. The objective of this investigation, which became possible through an initial inquiry into systemic lupus erythematosus in young patients, was to examine the association between lymphopenia and the clinical and biological manifestations and activity of lupus. The exclusion criterion for the study was individuals under the age of 16 who had been diagnosed with lupus over a period of 36 months (2015 - 2018). Lymphopenia was defined as a rate of less than 1,500/mm^3. The study included 83 eligible patients who were divided into two groups based on their lymphocyte count: one group with less than 1500/mm^3 (n = 47) and the other with greater than 1500/mm^3 (n = 36). The severity of lymphopenia was evaluated based on its depth: mild: [1000 - 1500 mm^3], moderate to severe: < 1000/mm^3. A comparative evaluation of the two groups was performed to assess the possibility of clinico-biological associations. The results showed a sex ratio of boys to girls of 1:4.9 (0.20), a mean age at diagnosis of 11.3 ± 3.62, and lymphopenia prevalence of 71% with a mean of 865.57 ± 294.12 [200 - 1400/mm^3], of which 32% was mild and 68% was moderate to severe. Comparison of the two groups revealed a significant correlation between lymphopenia and renal involvement (p = 0.02), neutropenia (p = 0.02), leukopenia (p = 0.0004), decreased C3 (p = 0.00006), C4 (p = 0.0009), macrophagic activation syndrome (p = 0.04), and disease activity (p = 0.025). In conclusion, lymphopenia was associated with certain severe clinical and biological forms and could be a practical and effective means of assessing the severity of juvenile lupus.

Keywords: *Childhood Systemic Lupus Erythematosus; Lymphopenia; Clinical Manifestations; Biological Abnormalities; Disease Activity*

Abbreviations

ACR: American College of Rheumatology; LN: Lupimanuke Nephropathy; MAS: Macrophagic Activation Syndrome; SLE: Systemic Lupus Erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics; pSLE: Pediatric-Onset Systemic Lupus Erythematosus; PRES: Posterior Reversible Encephalopathy Syndrome

Introduction

Lymphopenia, a biological abnormality, is a commonly occurring manifestation in systemic lupus erythematosus [1]. Its utility, however, has been restricted to the diagnostic criteria of lupus disease, specifically the American College of Rheumatology (ACR) and the Systemic Lupus International Collaborating Clinics (SLICC) [2]. As part of a thesis project on "Systemic lupus erythematosus in children: severity factors", a preliminary study was conducted at the N'Fissa Hamoud University Hospital in Algiers. The study aimed to ascertain the correlation between lymphopenia in SLE and clinical and immunological symptoms along with disease activity.

Materials and Methods

A prospective, descriptive, analytical, multicentre, multivariate study was conducted over a 36-month period (January 2015 - December 2018) that included patients under the age of 16 with systemic lupus erythematosus who met the criteria of the American College of Rheumatology (ACR) classification. Neonatal lupus and induced lupus were excluded from the study. To meet the objective of the study, a questionnaire was designed. Lymphopenia was defined according to ACR criteria as a lymphocyte count < 1500 cells/mm^3. Only lymphopenia attributable to SLE and not to a drug-related cause, particularly immunosuppressants and corticosteroids, or other causes, was considered. The lymphocyte count chosen was that at the time of diagnosis of pSLE. The individuals were grouped into two categories depending on their lymphocyte count: one category with lymphopenia (lymphocyte count < 1500/mm^3) and the other category without lymphopenia (lymphocyte counts > 1500/mm^3). The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was used to estimate disease activity, which is a widely used and validated tool for assessing SLE in children and adults. Activity levels and relapse criteria were clearly defined, with a score ≥ 20 indicating a very highly active lupus disease. The activity thresholds were determined based on the score range, with mild being between 6 - 10, moderate being between 11 - 20, and severe being greater than or equal to 20 (Table 1). Statistical analysis was performed using Epi Info™ and EpiData version 3.2 software. Two methods of statistical analysis were utilized: one was univariate analysis that employed percentages, means, and standard deviations, and the other was bi- and multivariate analysis that employed Pearson's Chi-square test, Student's t-test, and, in certain situations, Fisher's exact test. The statistical tests performed had an alpha significance level equal to 5% with a 95% confidence interval, and the Chi-square tests, means and percentages were specified.

SLEDAI score	Activity levels
= 0	No activity
[1-5]	Light activity
[6-10]	Medium activity
[11-19]	High activity
≥ 20	Very high activity

Table 1: *Activity levels according to the SLEDAI score.*

Results and Discussion

General data: The initial study consisted of 83 patients who all met the revised ACR criteria for the diagnosis of SLE. Out of these patients, 69 were female, with a sex ratio of 1:4.9 (0.20). The mean age at diagnosis was 11.3 ± 3.62, with a range of 2 - 16 years. Figure 1 displays the clinical and biological characteristics of these patients, with skin involvement being considered the "hallmark" of lupus and being the earliest and most consistent of the clinical features. Hemorrhagic involvement was highly frequent, with a prevalence of 79.5%, distributed as follows: Anemia (73%), lymphopenia (71%), neutropenia (50%), leukopenia (47%), and thrombocytopenia (35%); figure 2.

Constitutional signs were highly prevalent among initial clinical manifestations, with asthenia being the main symptom in 94.6% of cases, followed by weight loss and fever. Joint involvement was significant, with an incidence of 65% of cases in the form of non-erosive,

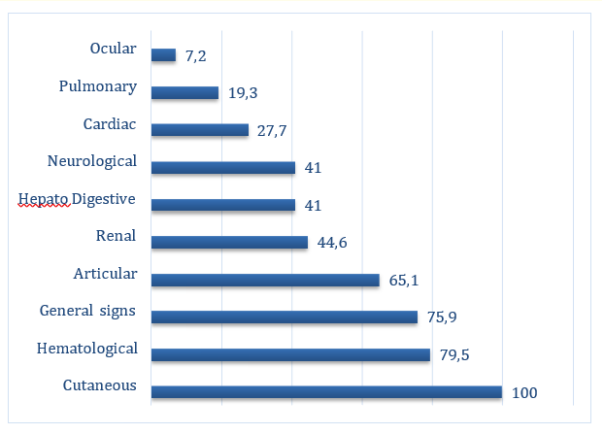

Figure 1: *Distribution of patients according to initial clinical involvement.*

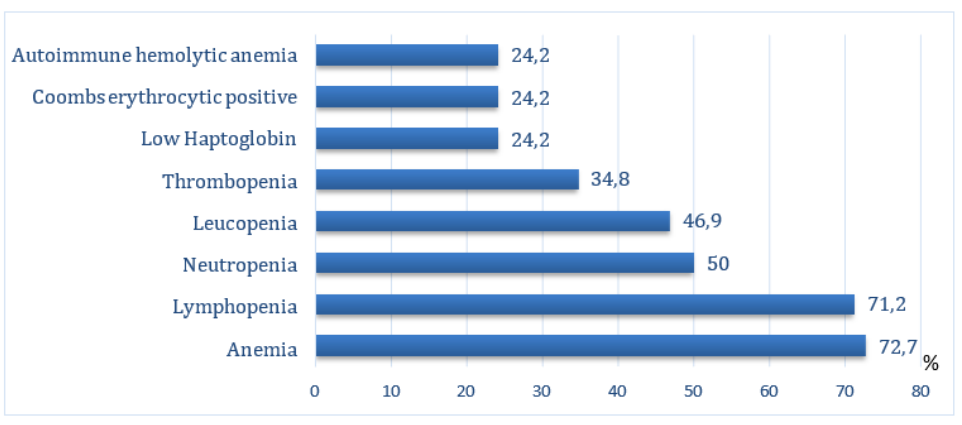

Figure 2: *Distribution of patients according to initial clinical involvement.*

grossly symmetrical, very painful polyarthritis, with 63% of patients suffering from a severe morning rash. Renal involvement was observed in 47% of cases, with the frequency of occurrence increasing over the course of the study, particularly in the first two years of lupus disease progression, ultimately reaching a prevalence of 51% by the end of the study. Renal biopsy was performed in 31/37 patients with renal manifestations, while in 6 patients, it was not conducted due to the rapidly fatal course.

Out of a total of 30 kidney biopsies interpreted (with only one being uninterpretable), diffuse endothelial proliferative forms (III, IV and VI) accounted for 73%. Pure mesangial forms (I and II) accounted for 27% of cases, according to the ISN 2003 classification of lupus nephropathies. Neurological involvement was present in 41% of cases, with headaches (38.2%), psychosis (38.2%), convulsions (35.3%), and motor deficits (32.4%). Other neuropsychiatric manifestations accounted for 32.4% of cases in the form of various neurological syn-

dromes: Charles Bell syndrome, PRES syndrome, polyradiculoneuritis, cranial pair involvement, cerebellar ataxia, encephalitis, peripheral neuropathy, Broca's aphasia, and aseptic meningitis.

Hepato-digestive involvement was highly frequent, occurring in 41% of cases, with abdominal pain accounting for 61.8% of cases, followed by acute hepatitis in 41.2% and pancreatitis in 14.7%. Hepatosplenomegaly accounted for 73.6% of cases, most often associated with other hepato-digestive disorders. Results and discussion must illustrate and interpret the reliable results of the study.

Cardiac manifestation was observed in 28% of the cases, predominantly characterized by pericarditis (69.9%), Libman-Sacks endocarditis (39.1%), myocarditis (4.3%), and heart failure (8.7%).

A comparison of two groups was conducted in this study, which consisted of 83 cases. Among the 83 cases compared in this study, it was observed that 47 patients had lymphopenia and the remaining 36 had a normal lymphocyte count. The mean lymphopenia was found to be 865.57 ± 294.12, with a range of extremes from 200 - 1400/mm^3. The depth of lymphopenia was assessed based on the lymphocyte count, which allowed for the identification of two distinct groups of patients. These groups ranged from those with mild lymphopenia (32%) to those with moderate to severe lymphopenia (68%), with a range of extremes from 200 elements/mm^3 to 975 elements/mm^3.

A comparative analysis of the two groups was performed to evaluate the potential clinico-biological association of this hematological factor. The findings of the present investigation, as portrayed in table 2 and 3, reveal a statistically significant correlation with renal impairment (p = 0.02) and macrophagic activation syndrome (MAS) (p = 0.04). Indeed, among the 18 patients with MAS in our series, 14 presented with severe lymphopenia. Although lymphopenia in SLE is considered an independent entity of other cytopenias, a significant association was found with leukopenia (p = 0.0004) and neutropenia (p = 0.02). Hypocomplementemia was also found to be closely linked to lymphopenia in C3 (p = 0.00006) and C4 (p = 0.0009) fractions.

	Total lymphocyte count < 1500 (cells/mm)		**Total lymphocyte count ≥ 1500 (cells/mm^3)**		**P**
	Number	**%**	**Number**	**%**	
Malar exanthema					
Yes	38	80,9	24	66,7	= 0.14
No	9	19,1	12	33,3	
Oral ulceration					
Yes	25	53,2	22	61,1	= 0.47
No	22	46,8	14	38,9	
Rheumatological conditions					
Yes	33	70,2	21	58,3	= 0.26
No	14	29,8	15	41,7	
Neurological impairment					
Yes	19	40,4	15	41,7	= 0.90
No	28	59,6	21	58,3	
Pleural effusion					
Yes	8	17	4	11,1	= 0.44
No	39	83	32	88,9	

Pericarditis					
Yes	9	19,1	7	19,4	= 0.97
No	38	80,9	29	80,6	
Kidney involvement					
Yes	26	55,3	11	30,6	= 0.02*
No	21	44,7	25	69,4	
Infections					
Yes	29	61,7	16	44,4	= 0.11
No	18	38,3	20	55,6	

Table 2: *Correlation of lymphopenia with clinical findings.*

	Total lymphocyte count < 1500 (cells/mm³)		**Total lymphocyte count ≥ 1500 (cells/mm³)**		**P**
	Number	**%**	**Number**	**%**	
Autoimmune hemolytic anemia					
Yes	11	23,4	5	13,9	= 0.27
No	36	76,6	31	86,1	
Thrombocytopenia					
< 100.000	10	21,3	7	19,4	= 0,83
≥ 100.000	37	78,7	29	80,6	
Leukopenia					
< 4000	27	57,4	7	19,4	= 0.0004*
≥ 4000	20	42,6	29	80,6	
Neutropenia					
< 2000	25	53,2	10	27,8	= 0.02*
≥ 2000	22	46,8	26	72,2	
Macrophagic activation syndrome					
Yes	14	29,8	4	11,1	= 0.04*
No	33	70,2	32	88,9	
ANA					
≥ 1/1000	25	53,2	24	66,7	= 0.21
< 1/1000	22	46,8	12	33,3	
Anti DNA antibodies					
Yes	37	78,7	25	69,4	= 0.33
No	10	21,3	11	30,6	
Anti Sm antibodies					
Yes	23	48,9	16	44,4	= 0.68
No	24	51,1	20	55,6	

Anti SSA antibodies					
Yes	18	38,3	15	41,7	= 0.75
No	29	61,7	21	58,3	
APL					
Yes	23	48,9	20	55,6	= 0.54
No	24	51,1	16	44,4	
Complement component 3 (C3) Low					
Yes	35	74,5	11	30,6	= 0.00006*
No	12	25,5	25	69,4	
Complement component 4 (C4) Low					
Yes	34	72,3	13	36,1	= 0.0009*
No	13	27,7	23	63,9	

Table 3: *Correlation of lymphopenia with biological and immunological abnormalities.*

Results of the present investigation indicate a significant association between high to very high activity levels and lymphopenia (p = 0.025) in SLE patients. In fact, 33 out of 47 patients with lymphopenia had a SLEDAI score ≥ 20, indicating high activity, as presented in table 4.

SLEDAI score	Total lymphocyte count < 1500 (cells/mm^3)		Total lymphocyte count ≥ 1500 (cells/mm^3)		P
	Number	%	Number	%	
[1-5]	0	0	4	11	0.025* statistically significant
[6-10]	8	17	7	19	
[11-19]	6	13	9	25	
≥ 20	33	70	16	45	

Table 4: *Correlation between SLEDAI activity levels and lymphopenia.*

Pediatric-onset systemic lupus erythematosus (pSLE) is a chronic autoimmune disease that exhibits significant clinical and biological polymorphism. It is distinguished by its severity in comparison to adult forms [3]. Despite this, few studies have focused on pSLE, and even fewer on lymphopenia. Lymphopenia is a frequent manifestation of pSLE, and its implications extend beyond diagnostic interest.

Our series of patients revealed that 71% exhibited lymphopenia, a prevalence comparable to that of two major studies of juvenile SLE. Hoffman's cohort (56 patients) exhibited a rate of 67.9% [4], while Watson's cohort (198 patients) exhibited a rate of 73% [3].

We were able to demonstrate that lymphopenia is statistically linked to certain clinical manifestations, such as renal impairment. Specifically, of the 37 patients with lupimanuke nephropathy (LN), 26 (70%) had lymphopenia, and their LN was class III or IV. This association is the result of a complex dynamic between lymphocytes and kidney cells. In lupus nephropathy, T lymphocytes flock to the site by chemotaxis, directly involving them in the recruitment of macrophages, which are considered the main effector cells in lupus nephritis. Additionally, T lymphocytes stimulate cytokine production, which has pro-inflammatory activity and modulates vascular permeability [5].

T lymphocytes may also have a direct cytotoxic effect on kidney cells, which may explain their consumption by high activation during active SLE, particularly in advanced LN. Other studies have demonstrated an increase in anti-T-cell antibodies in patients with particularly proliferative lupus glomerulonephritis, which may explain the lymphopenia in active LN [6].

The correlation between lymphopenia and MAS in pediatric lupus has not previously been described. MAS is responsible for the activation of several immunocompetent cells, resulting in cytopenia, including lymphopenia. In addition to the consumption of cells by phagocytosis, there is also a depletion of myeloid precursors, reflecting the suppressive action of cytokines such as INFγ, TNF-α and IL1, which contribute to cytopenia and lymphocyte imbalance. As a result, it is difficult to attribute lymphopenia to SLE alone, given the pathogenic similarity between secondary MAS in general and SLE. Both pathologies involve various cells and cytokines implicated in cytopenia and particularly lymphopenia.

Hypocomplementemia is a recognized indicator of potential inflammation that is associated with the consumption of complement by tissue-bound immune complexes [7]. Multiple studies have established a strong correlation between decreased complement levels (C3 and C4) and lupus activity [7], particularly during renal flares. Our study has clearly demonstrated a significant association between lymphopenia and NL, and its connection with a decrease in C3 and C4 can be explained. Our findings are in line with N Sobhy., *et al*.'s study, which included 124 patients and demonstrated the correlation between lymphopenia and hypocomplementemia [8].

Regarding the correlation between lymphopenia and disease activity (SLEDAI) in our study, we have observed that 39 out of 47 patients with lymphopenia had SLEDAI activity levels ≥ 11, including 33 cases with high activity ≥ 20 (Table 4). This association with disease activity may be explained by a variety of factors linked lymphocyte depletion during active lupus.

Dhir., *et al*. confirmed the presence of a higher degree of apoptosis in T lymphocytes in patients with SLE, discovering a direct correlation between T lymphocyte apoptosis and disease activity [9]. In the study by Rastin., *et al*. several immunological agents were shown to be involved in T-cell apoptosis during active lupus disease [10]. Indeed, an increase in the expression of the death receptor Fas, its ligand (FasL), and caspase-8, which are essential for the transduction of apoptosis signals in the extrinsic pathway, could explain the decreased lymphocyte count [11].

In other words, high expression of the membrane-bound and soluble Fas antigen (CD95/Apo-1), an apoptosis inducer belonging to the Tumor Necrosis Factor Receptor TNF-R family also known as CD95 Apo 1, leads to increased T-cell apoptosis. This antigen and its ligand are thought to contribute to pathogenesis by overprotecting lymphocyte apoptosis receptors, resulting in increased lymphocyte death. Furthermore, the depletion of the CD28 molecule, which is regarded as a potent co-stimulatory signal for T cell activation, in conjunction with the depletion of CD4 and CD8 T cells in lupus patients, is believed to increase the vulnerability of T cells to cell death and may therefore be involved in lymphopenia [12].

The correlation between lymphopenia and the clinical manifestations of SLE, specifically renal involvement and disease activity, has been documented in a limited number of studies in adults. Notably, Vila's American study of 591 patients, Faddah's Egyptian study of 30 patients, and the cohort of F, Ha-Ou-Nou., *et al*. comprising of 148 patients [13-16]. On the contrary, there are scarce findings available in the literature regarding lymphopenia and its prognostic role in pediatric patients [17]. Our study elucidated several clinical and biological implications of lymphopenia, revealing it to be a predictive factor for active lupus. Furthermore, our study demonstrated that the depth of lymphopenia is independently correlated with SLE activity and severe visceral damage.

Conclusion

Lymphopenia is a prevailing phenomenon in systemic lupus erythematosus and is intricately associated with numerous severe clinical and biological disorders. Apart from its diagnostic significance, it could potentially be suggested as a predictive and prognostic factor for active relapses.

Conflict of Interest

Any conflict of interest exists.

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