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PREDICTIVE FACTORS OF DEPRESSION AND ANXIETY AND THEIR IMPACT ON THE OUTCOME OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

SUMMARY

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Motto

"The good physician treats the disease; the great physician treats the patient who has the disease."

Sir William Osler (1849-1919)
the father of modern medicine,
co-founder Johns Hopkins School of Medicine

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic, autoimmune condition that is extremely clinically heterogeneous with a complex, insufficiently elucidated pathogenesis. Immune system dysregulation induces dysfunction in multiple organs and systems, including the central nervous system.

Neuropsychiatric lupus is one of the most severe and prevalent manifestations of the disease, involving well-defined and classified neurological and psychiatric manifestations according to the American College of Rheumatology criteria.

Among neuropsychiatric manifestations, depression and anxiety are among the most prevalent, appearing much more frequently in patients with SLE compared to the general population or other connective tissue diseases. In addition to their significant frequency, depression and anxiety add to the devastating impact of the underlying disease, dramatically altering patients' quality of life, exacerbating the degree of physical and social disability, and increasing the risk of premature mortality and even suicide.

However, the exact prevalence of depression and anxiety among patients with SLE is not known, with published studies indicating different, discrepant reports depending on the scales and definitions used. Additionally, considerable efforts are being made to understand the exact pathogenesis of neuropsychiatric manifestations in particular and the etiopathogenesis of lupus disease in general for a more specific, targeted diagnostic and therapeutic approach, ideally personalized for patients who are so severely affected by this complex, multisystemic disease.

What is known so far about the predictive factors of depression and anxiety in lupus disease is that prednisone doses (≥ 20 mg/day) are an independent risk factor for their occurrence. Regarding disease manifestations, active skin lesions and neurological lesions such as myelitis are predictive factors for depression, while the global activity of the disease is not a risk factor for it. However, the severity of anxiety is independently associated with the activity of lupus disease.

Conversely, the progression of systemic lupus erythematosus can be impacted by the presence of depression and anxiety, as it is known that negative emotions influence the immune system through the sympathetic nervous system and endocrine system, inducing immune dysregulation through neuropeptides and hormones that can exert a direct pathogenic role in triggering lupus activity.

There is certainly a bidirectional relationship between the progression of lupus disease and these affective disorders, with SLE patients having twice the risk of developing depression and anxiety, although the exact substrate of these disorders in SLE is not known. However, there is already a demonstrated major interrelationship between psychiatric disorders and chronic somatic diseases.

Possible pathogenic mechanisms of neuropsychiatric involvement, already described in the literature, involve a predisposed genetic background, the presence of blood-brain barrier dysfunction, ischemic cerebrovascular lesions associated with inflammation induced by autoantibodies, complement activation, multiple cytokine involvement, thus outlining a dual pathogenic model, ischemic and neuroinflammatory, which constituted the starting point of my doctoral thesis.

As novel elements in the field, this study conducted the first research on the prevalence of depression and anxiety in SLE patients in Romania and the progression of depression and anxiety over one year. It conducted the first feasibility assessment of the WHODAS disability scale in SLE patients and its statistical validation for this pathology, provided data on possible pathogenic mechanisms by concomitant research on the impact of biomarkers associated with inflammation and thrombosis on the progression of depression and anxiety in SLE patients, and correlated the status of depression and anxiety with the progression of lupus disease over one year in terms of activity and irreversible damage.

This study is an advocacy for the proactive evaluation of debilitating symptoms of depression and anxiety in patients with systemic lupus erythematosus. It also highlights the need for more rigorous longitudinal studies to identify specific serological markers for neuropsychiatric lupus. These markers would facilitate early diagnosis and treatment, thereby improving the quality of life and prognosis of these patients.

GENERAL PART
SYSTEMIC LUPUS ERYTHEMATOSUS AND NEUROPSYCHIATRIC LUPUS
CURRENT STATE OF KNOWLEDGE

Systemic lupus erythematosus (SLE) is an autoimmune condition with a diverse clinical spectrum and an etiology composed of genetic, epigenetic, ethnic, immunoregulatory, hormonal, and environmental factors. This pathology manifests through systemic inflammation and the production of autoantibodies, with varied phenotypes ranging from mild cutaneous and mucosal symptoms to severe complications affecting multiple organs. (1)

Even though significant progress has been made in technological development and understanding the pathophysiological bases and risk factors of systemic lupus erythematosus, the exact mechanisms underlying the development of the disease remain partly unexplained. Establishing the diagnosis of SLE can be challenging, and the validity of the proposed classification criteria continues to be a topic of debate in clinical practice. (2)

A precise and rapid diagnosis is crucial to initiating appropriate treatment and preventing disease complications. The management of SLE depends on the severity of organ involvement and often requires a multidisciplinary approach. (4)

The evolutionary dynamics of SLE vary, with approximately 70% of patients presenting a relapsing-remitting course of the disease, while the rest are divided between prolonged remission and persistent active disease. Treatment aims at long-term survival, preventing exacerbations and organ damage, and improving the quality of life of patients. (3)

Functional deregulation of the immune system can cause dysfunction in a variety of organs and systems, including the central nervous system (CNS). Neuropsychiatric complications associated with systemic lupus erythematosus (NPSLE) represent a severe manifestation of the disease, with a wide range of neurological and psychiatric symptoms that develop as a direct consequence of SLE. (6)

The symptoms of NPSLE are variable, potentially being focalized or generalized, affecting both the peripheral and central nervous systems, with severity levels ranging from mild to severe. (7,8)

Diagnosing NPSLE is difficult for clinicians, especially for rheumatologists, due to the absence of specific and sensitive laboratory tests, biological markers in cerebrospinal fluid (CSF), conclusive radiological imaging evidence, or well-established diagnostic criteria to guide the treatment and management of this clinical entity. (6)

Like the pathogenesis of SLE, that of NPSLE is multifactorial, complex, involving a variety of inflammatory cytokines, genetic factors, multiple autoantibodies, blood-brain barrier dysfunction, complement activation, and immune complex deposition. These elements contribute to vasculopathy, cytotoxicity, and neuronal damage mediated by autoantibodies, detailed in the specialized literature.

Although several mechanisms have been described in the literature, the pathological processes leading to neurological damage, pathophysiological changes, and consequently, the clinical manifestations in SLE patients are still insufficiently elucidated and require further research for a complete understanding.

Blood-brain barrier (BBB) deterioration is a critical element in the neuropsychiatric pathogenesis of systemic lupus erythematosus. Two fundamental mechanisms identified in subsequent research are proposed as causes of its dysfunction,respectively the autoimmune or inflammatory pathway and the ischemic or thrombotic pathway, recognized as fundamental in the pathogenesis of NPSLE. On one

hand, the autoimmune or inflammatory pathway contributes to neuropsychiatric manifestations through inflammatory mediators or autoantibodies, or through the formation of immune complexes that disrupt BBB integrity. On the other hand, the ischemic or thrombotic pathway is characterized by cerebral microangiopathy, vascular occlusion, and hemorrhage. This latter pathway is associated with accelerated atherosclerosis and immune-mediated vascular lesions, which can lead to a variety of neuropsychiatric manifestations in NPSLE. Understanding these pathogenic processes is crucial for developing effective therapeutic strategies for NPSLE. (182, 183, 184)

The pathogenesis of NPSLE is complex and unclear, but the two pathways: ischemic and neuroinflammatory, remain fundamental and are most likely complementary, as described by Minhuin Wang in a review article published in 2022, and as indicated by the schematic representation of the pathogenesis in Figure 1. (185)

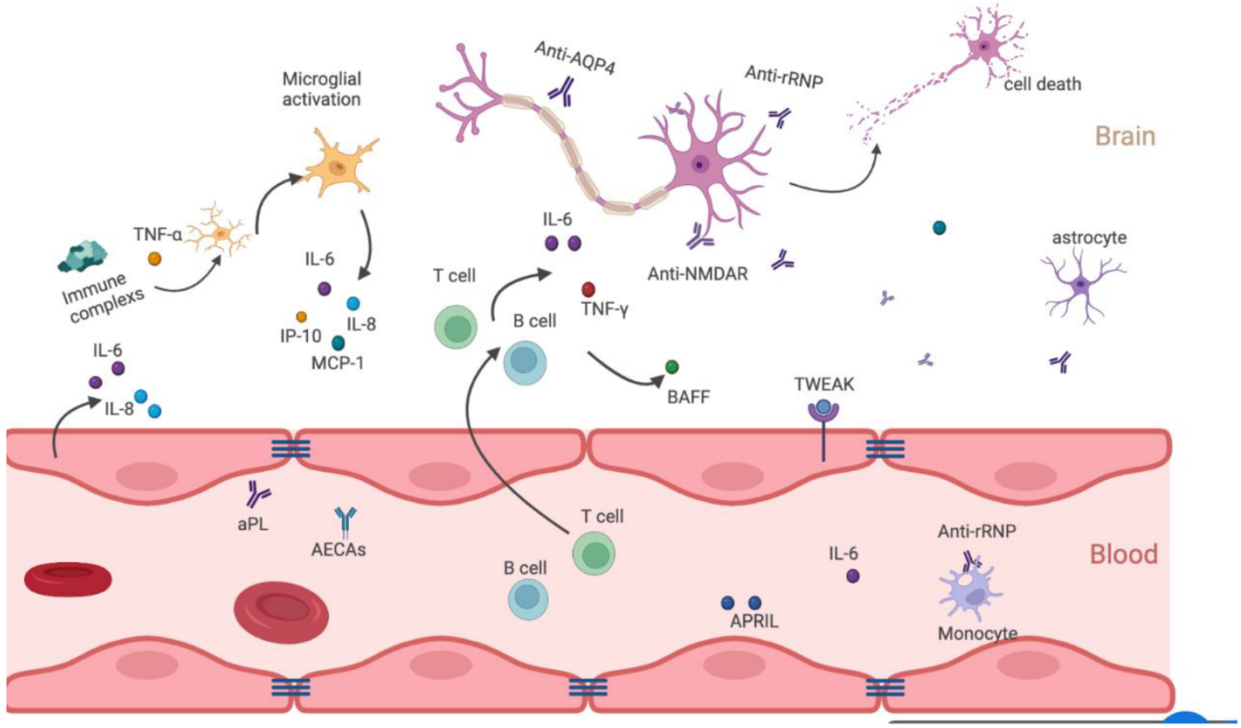


Figure 1. Pathogenic Mechanisms of diffuse NPSLE (adapted from Wang, M.J. Clin. Med. 2022, 11, 4955.)

BBB dysfunction is a significant pathogenic characteristic not only in systemic lupus erythematosus but also in other neurological conditions such as Alzheimer's disease, multiple sclerosis, and stroke. (186,187) This dysfunction is particularly correlated with the systemic inflammatory response, manifesting through the overexpression of pro-inflammatory cytokines like IL-1β, IFN-γ, and IL-6, which facilitate the infiltration of immune cells into the central nervous system (CNS). IL-1β, in particular, plays an essential role in inducing the expression of adhesion molecules, thus facilitating leukocyte adherence and transmigration into the BBB.

In a pathological context, microglia can adopt a specific inflammatory profile that contributes to the progression of neurodegenerative diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, and multiple sclerosis. These cells, along with other resident brain cells like astrocytes and endothelial cells, can play an active role in promoting chronic inflammation and neurodegeneration. (216,217)

Studies conducted on post-mortem brain tissues from individuals with NPSLE have provided valuable insights into the links between brain lesions and cerebrovascular lesions associated with this condition. (7) Cerebral ischemia and microvascular thrombosis, along with non-inflammatory vascular lesions and microhemorrhages, are often observed pathological events in the context of NPSLE. A consistent association has been reported between the deposition of complement components and specific pathological lesions of NPSLE, with microthrombi associated with C4d and C5b-9 accumulations detected exclusively in NPSLE. These data indicate a key role of the complement system in the interaction between circulating autoantibodies and NPSLE-associated lesions, highlighting the potential of complement inhibition strategies in the treatment of the condition. (182) Immune complexes, complement activation, and autoantibody-mediated vascular impairment are central factors in the etiopathogenesis of NPSLE, with demonstrable roles in the clinical development of the disease. (183,229)

Recent discoveries about the mechanisms involved offer perspectives for improving treatment strategies for neuropsychiatric symptoms associated with SLE. Direct CNS lesions can result from amino acid toxicity, oxidative stress, inhibition of plasminogen activator inhibitor (PAI-1) activity, and matrix metalloproteinase 9 (MMP-9) activity. (230) Direct brain lesions in NPSLE can result from mechanisms such as amino acid toxicity, oxidative stress, inhibition of plasminogen activity (PAI-1), and MMP-9 activity. (231,232,233)

These factors can induce neuronal damage through microglia activation and promotion of neuronal apoptosis, contributing to diffuse manifestations of NPSLE, including acute confusion and psychosis. (7,231) The production of autoantibodies is a central element in the development of lupus pathogenesis, being closely associated with tissue damage and organ dysfunction observed in systemic lupus erythematosus. (46) These autoantibodies are identifiable in the vast majority of patients, with an incidence of 90-95%. (234)

High titers of antinuclear antibodies are often detected in individuals with systemic neuropsychiatric manifestations of lupus. Even though ANA are important in the evaluation and understanding of NPSLE pathogenesis, studies indicate that using ANA as a screening tool during the first psychiatric episodes is not always specific for diagnosing this condition. This is partly attributed to the frequency of false-positive results, which can occur in the context of certain drug treatments. (238,239)

Starting from 2019, the joint recommendations of the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) established that a minimal criterion for diagnosing systemic lupus erythematosus (SLE) is an antinuclear antibody (ANA) titer of ≥1:80 or a positive result in an equivalent test, thus confirming ANA testing as an efficient and sensitive screening tool for SLE. (234) Even though more than 100 autoantibodies have been identified in individuals with SLE or lupus-associated neuropsychiatric manifestations, the exact role of these autoantibodies in the complex development of NPSLE has not yet been definitively established. (240)

Among the mentioned antibodies, a major pathogenic role belongs to antibodies associated with the antiphospholipid syndrome, with the CNS being particularly susceptible to thrombosis, and the presence of aPL antibodies associating with an increased risk of ischemic stroke (261) and accelerated atherosclerosis. (262) Besides the thrombotic risk, aPL antibodies are also linked to various manifestations of systemic lupus erythematosus with neuropsychiatric involvement (NPSLE), including seizures, abnormal movements like chorea, cognitive problems, and myelopathy (263,264,265) and especially with psychosis. (266,267,268)

Recent data indicate that antiphospholipid (aPL) antibodies might contribute to neuronal damage by generating oxidative stress and affecting neuronal membranes, involving β2-glycoprotein. Laboratory studies have demonstrated the ability of aPL antibodies to bind to neurons and induce hyperactive behavior when administered directly into the central nervous system of laboratory animals. (269) These findings suggest a neuroinflammatory effect of aPL antibodies, which may play a role in the thrombotic and neuroinflammatory pathogenesis in SLE patients, contrary to the previous opinion that the procoagulant state mediated by aPL is non-inflammatory. Additionally, research has shown that mice deficient in components of the complement system C3 and C5 are protected from aPL-mediated thrombosis and endothelial activation. (270)
Complement activation is correlated with specific manifestations of focal NPSLE, cognitive disorders, and psychosis, suggesting a significant inflammatory contribution to these conditions. (271) It is believed that many of the neurological symptoms associated with antiphospholipid antibodies result from ischemic events in essential brain areas such as the amygdala, hippocampus, and frontal cortex. (244) Additionally, vascular lesions caused by thrombosis can compromise the blood-brain barrier, thereby facilitating the entry of peripheral inflammatory factors into the CNS, including circulating pathogenic antibodies and leukocytes, amplifying brain damage. (272,273)
The presence of antiphospholipid antibodies in individuals with systemic lupus erythematosus doubles the risk of developing neuropsychiatric forms of the disease compared to patients without aPL. (179) Accumulated evidence from studies shows that these antibodies constitute a significant risk factor for NPSLE, emphasizing their importance in evaluating and managing patients with SLE. (274,220)
Inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), TWEAK (TNF-like weak inducer of apoptosis), interferon-gamma (IFN-γ), interleukin-6 (IL-6), interleukin-8 (IL-8), and B-cell activating factor (BAFF), have been found in the cerebrospinal fluid of patients with NPSLE. This indicates a major role of inflammation in the evolution of NPSLE. (309)
In conclusion, neuropsychiatric systemic lupus erythematosus is a condition characterized by a complex and still insufficiently explained pathogenesis. Notable progress has been made recently in elucidating this condition by evaluating a wide range of biomarkers and autoantibodies. Future investigations are expected to bring further clarifications on the pathogenic and pathophysiological mechanisms involved in NPSLE, thus paving the way for innovative treatments, with specific action on the causes of the disease, which could improve the survival rate and quality of life of patients. (175)

CLINICAL MANIFESTATIONS, DIAGNOSIS, AND MANAGEMENT OF NPSLE

Neuropsychiatric manifestations of SLE (NPSLE) represent a major manifestation of SLE that involves the nervous system, generating neurological or psychiatric symptoms that can lead to a significant decrease in quality of life, alter the vital prognosis, and are associated with high mortality. (330, 331) NPSLE can appear as the first manifestation of the disease. (309)
This condition affects both the central and peripheral nervous systems, as well as the autonomic nervous system, with a spectrum of symptoms ranging from subtle changes to severe problems such as headache, cerebrovascular lesions, cognitive disorders, epilepsy, and acute disturbances of consciousness. (332) Conditions involving the central nervous system are more common than those of the peripheral nervous system. (333)
According to the American College of Rheumatology criteria, NPSLE is characterized by a range of 12 neuropsychiatric symptoms associated with the central nervous system and seven associated with the peripheral nervous system, plus autonomic nervous system neurological syndromes. However,

certain neuropsychiatric syndromes that may occur in SLE, such as small fiber neuropathy, chronic inflammatory demyelinating polyneuropathy, reversible posterior encephalopathy syndrome, and neuromyelitis spectrum disorders, are not included in this classification. (334)

Table 3. Clinical manifestations in NPSLE (adapted from: The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes, 1999)

Central nervous system		Peripheral nervous system
Focal symptoms	Diffuse symptoms	
Aseptic meningitis	Cognitive dysfunction	Guillain-Barre syndrome
Cerebrovascular disease	Mood disorder	Autonomic disorder
Demyelinating syndrome	Anxiety disorder	Mononeuropathy
Headache	Acute confusional state	Myasthenia gravis
Movement disorder	Psychosis	Cranial neuropathy
Myelopathy		Plexopathy
Seizure disorder		Polyneuropathy

Often, up to half of individuals with SLE develop NPSLE during the course of the disease, with increased frequency in the first 3-5 years from the initial diagnosis. (335) Headache, depression, anxiety, and cognitive dysfunction are among the most common symptoms of NPSLE. Studies suggest that ethnicity and advanced age may contribute to the earlier onset of neuropsychiatric lesions. (309)

When evaluating patients diagnosed with SLE who present new or aggravated symptoms that may indicate neuropsychiatric conditions, the initial approach is similar to that applied to patients without SLE who manifest equivalent symptoms. The first step is to rule out other possible causes, such as infections, metabolic or endocrine dysfunctions, and potential side effects or adverse reactions to medications. (220)

Affective and Anxiety Disorders

The ICD-10 (International Classification of Diseases 10th Revision) categorizes a depressive episode based on two main groups of symptoms: one characteristic and the other common, thereby establishing the severity of the episode into mild, moderate, and severe forms, with flexible criteria for determining intensity. (418) Main symptoms include: presence of a deep state of sadness, loss of interest or pleasure in usual activities, marked fatigue, and low energy levels.

The ICD-10 classifies generalized anxiety disorder under code F41.1. This condition is characterized by persistent and excessive worry that negatively affects daily functioning. (418)
There is no single test with complete sensitivity and specificity for diagnosing NPSLE. A proper diagnostic approach requires a thorough evaluation that includes rheumatologic examination, imaging, serologic tests, psychiatric and neuropsychological assessments, under the supervision of an interdisciplinary team comprising rheumatologists, neurologists, psychiatrists, and psychologists. (334) Differentiating between functional and organic causes of psychiatric symptoms is often challenging. (420)
Depression is the most common mood disorder associated with NPSLE, with an estimated lifetime prevalence of 65%. (421) In comparison, manic manifestations are significantly less common. A twofold higher rate of depression has been observed in patients with lupus compared to the general population. (421) Anxiety disorders have also been reported with greater frequency among individuals with SLE compared to healthy individuals. (49)
Recent research indicates a variable prevalence of depression and anxiety among patients with systemic lupus erythematosus (SLE), ranging from 2% to 91.7% in different studies. (422,423) This variation in study reports is likely influenced by multiple factors, including research methodology, definitions used for depression and anxiety, demographic characteristics of the studied population, and diverse screening techniques. (424)
Given the high prevalence of neuropsychiatric disorders, periodic neuropsychological evaluations are recommended alongside the routine assessment of lupus to identify neuropsychiatric disorders.

Treatment of NPSLE

Neuropsychiatric symptoms in individuals diagnosed with NPSLE present a wide variety, and due to an incomplete understanding of its etiology, specific therapeutic options are limited. The 2010 EULAR recommendations suggest a pragmatic approach for managing these manifestations, similar to the treatment of patients without SLE. Thus, the initial priority is symptomatic treatment, which includes correcting blood pressure and metabolic disorders, using antiepileptics in case of seizures, and administering anxiolytics, antidepressants, mood stabilizers, or antipsychotics, as needed, for psychiatric manifestations. Simultaneously, treatment for SLE should be adapted according to the origin of the neuropsychiatric symptoms, whether it is a diffuse syndrome caused by inflammation or the result of a focal thromboembolic process. (220)

PERSONAL RESEARCH AND ACHIEVEMENTS

OBJECTIVES OF THE WORK

Primary Objective:

Establishing the prevalence of depression and anxiety in patients with SLE.

Secondary Objectives:

1. Correlating the severity of depression and anxiety with the presence of coagulation or inflammatory disorders.
2. Impact of the presence of depression and anxiety on the evolution of lupus over a 12-month period.
3. Correlating the therapeutic response of depression and anxiety with the evolutionary status of SLE.

RESEARCH MATERIALS AND METHODS

A longitudinal study included adult outpatients diagnosed with SLE according to the updated 1997 ACR criteria (322) or the validated 2012/2019 SLICC classification criteria (235) at least six months before inclusion. Patients were recruited from June 2019 to January 2020 and followed for 12 months. The study was conducted within the Allergy-Immunology Clinic, Internal Medicine III Section of the County Emergency Clinical Hospital Brașov, after approval by the local Ethics Committee (approval number 19/22.03.2019). All patients provided written informed consent to participate in the study. All procedures were performed according to local regulations. The duration of the disease was considered from the moment of meeting the diagnostic criteria for SLE.

To ensure consistency and uniformity of the researched data, we established inclusion and exclusion criteria for this study as follows:

Inclusion Criteria:

1. The patient is at least 18 years old.
2. The patient was diagnosed with SLE at least 24 weeks before the initial visit, meeting 4 of the 11 Revised Criteria for the Classification of Systemic Lupus Erythematosus according to the 1997 update for ACR 1982 (Tan et al. 1982; Hochberg et al. 1997) or at least 4 of the 2012 SLICC criteria (Petri et al. 2012), including at least 1 clinical criterion and 1 immunologic criterion.
3. The patient is able to read, understand, and provide written informed consent. All procedures will be performed after signing the informed consent approved by the ethics committee.
4. Patients on standard background therapy, at least one of the following: a. Prednisone or equivalent – maximum 20 mg/day, at least 4 weeks before, stable dose at least 2 weeks before inclusion. b. Any of the following medications, administered for at least 12 weeks before signing the ICF and at a stable dose for at least 8 weeks before signing the ICF:
    - Azathioprine – maximum 200 mg/day

- Antimalarials (chloroquine, hydroxychloroquine, quinacrine) maximum dose 400 mg/day
- Mycophenolate mofetil – maximum 2 g/day OR mycophenolic acid – maximum 1.44 g/day
- Methotrexate (oral, SC, or IM) – maximum 25 mg/week

5. Lupus disease is not clinically and biologically active according to recommended scales (SLEDAI assessment <4 points, no BILAG A score or >2 BILAG B scores).

Exclusion Criteria:

1. Any disease that, in the physician's opinion, would interfere with study evaluations or data and result interpretation (including history of neoplasia, primary psychiatric disorders, severe chronic infections, severe renal, hepatic, cardiac diseases).
2. Concurrent enrollment in another clinical study using an investigational product or modification of the standard SLE background treatment scheme during the evaluation period.
3. Current or recent (less than 1 year before inclusion) alcohol, drug, or chemical substance abuse.
4. Active, severe, or unstable neuropsychiatric SLE (aseptic meningitis, cerebral vasculitis, demyelinating syndrome, acute myelopathy, acute confusional state, psychosis, acute inflammatory demyelinating polyneuropathy, status epilepticus, cerebellar ataxia).
5. Patients with rheumatoid arthritis, systemic scleroderma, primary Sjogren's syndrome, or other connective tissue diseases.
6. Patients with primary central nervous system pathology or other conditions that may induce cortical atrophy, stroke.
7. Patients with a history of epilepsy except for febrile seizures in childhood.
8. Pregnancy or breastfeeding.

After careful evaluation and adherence to the above criteria, the final research sample included 65 patients diagnosed with SLE, who were assessed over a 12-month period following a prospective and longitudinal model. Demographic data were collected from all patients, including age, sex, education, employment status (active/inactive/retired), marital status, smoking and alcohol consumption, personal physiological and pathological history, background therapy, and comorbidities with a focus on classical cardiovascular risk factors, dyslipidemia, diabetes mellitus (DM), and primary hypertension (PHT). Patients underwent a comprehensive clinical evaluation, including a complete physical and biological examination with serological determinations for SLE.

The evaluation also investigated inflammation status, complete blood count (CBC), renal function, complement levels (C3 and C4), antinuclear antibody profile (anti-dsDNA, anti-Sm, anti-histone, anti-Ro, anti-ribosomal P protein, anti-RNP), tests for inflammation and coagulation, aiming to cover the two pathogenic mechanisms described for other NPSLE determinations.

The instruments used to assess SLE activity were the British Isles Lupus Assessment Group 2004 index (BILAG 2004 index) and the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2K), and the SFI flare index. Only patients without disease activity were included.

Quality of life was assessed using validated tools for SLE: EQ-5D-5L (Euro Qol Dimensions), C-SSRS (Columbia-Suicide Severity Rating Scale), functional impact with the World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0).

Anxiety and depression were determined using standardized scales:

- Hamilton Anxiety Rating Scale (HAM-A) and the 17-item version of the Hamilton Depression Rating Scale (HAM-D17) (see annexes no. 9, 10). Depression is defined by a score equal to or greater than 8: 8-17 mild depression, 18-25 moderate depression, >26 severe depression. (445)
- Anxiety is defined as mild: 8-14, moderate: 15-23, and severe anxiety ≥24. (446) The degree of disability was assessed using the WHODAS 2.0 scale. (499,500)

WHODAS 2.0 measures average functioning in daily situations over the last 30 days and evaluates six domains of functioning: (1) cognition (understanding and communicating), (2) mobility (moving and getting around), (3) self-care (e.g., hygiene, dressing, and eating), (4) getting along with others, (5) life activities (ability to manage daily responsibilities), and (6) participation in society. (504) To analyze the degree of disability, thresholds based on ICF International Classification of Functioning percentages were used: absent (0-4%), mild (5-24%), moderate (25-49%), severe (50-95%), and extreme (96-100%). (505)

The procedures performed at the initial inclusion visit, at three months, six months, and the final visit at 12 months are detailed in the table below (Table no. 4).

Table no.4 Study procedures

Procedures	Initial visit	3 month visit	6 month visit	Final,12 month visit
Informed Consent	x			
Medical History, including past medical history (PMH)	x			
Cardiovascular Risk Factors	x			x
Background and Concurrent Therapy	x	x	x	x
Complete Physical Examination, Vital Signs	X			x
Weight, Height, BMI	X			x
Questionnaires: EQ-5D, Pain VAS, C-SSRS, WHODAS 2.0	X	x	x	x
Scales: HAM-D, HAM-A	X	x	x	x
Immunologic Profile: (anti-dsDNA, anti-Sm, anti-Ro, anti-histone, anti-RNP, anti-ribosomal P protein)	X			x
PAI-1, sICAM-1, Fibrinogen, anti-cardiolipin (IgG, IgM), anti-beta 2GPI (IgM, IgG), LA, High Sensitivity C-Reactive Protein (hsCRP), D-dimer	x			
C3, C4, ESR, ANA	x			x
CBC, Biochemistry, Proteinuria	x			x
Lipid Profile	x			x

SLEDAI-2K, BILAG 2004, SFI, SLICC-ACR	x	x	x	x

The exploration of possible vascular pathogenesis associated with NPSLE was conducted by determining the antiphospholipid syndrome profile, detecting anticardiolipin antibodies (isotypes IgG, IgM, anti-beta 2GPI [isotypes IgM, IgG]) using the ELISA method, and the presence of lupus anticoagulant using the coagulometric method.

To cover vascular mechanisms beyond the well-known determinants, ELISA was used, with Elabscience reagents, to determine intercellular adhesion molecule 1 (ICAM-1), plasminogen activator inhibitor-1 (PAI-1), and P-selectin. These determinations added novel elements to the current research, scarcely explored in the literature.

According to the study design, patients were monitored for 12 months regarding the clinical and biological evolution of lupus, the evolution of depression and anxiety. At the end of the monitoring period, cardiovascular risk factors, background and concurrent therapy, complete physical examination, vital signs, HAM-D, HAM-A scales, EQ-5D questionnaires, Pain VAS, C-SSRS, WHODAS 2.0, and paraclinical assessments were reevaluated through immunological profile (detecting specific SLE autoantibodies mentioned above), complement fractions, CBC, biochemistry, proteinuria with recalculation of activity and irreversible damage scores: SLEDAI-2K, BILAG 2004, SFI, SLICC-ACR.

Statistical Analysis

For data analysis, we used the IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY, USA: IBM Corp. We determined the Pearson correlation coefficient for pairs of studied variables, and correlation coefficients and p-values were calculated with a standard confidence interval of 95%. The significance level was set at p-values less than or equal to 0.05. For univariate comparison between categorical variables, we used the chi-square test. Since some data did not show a standard distribution, we opted for a non-parametric test, and for continuous variables, we used the Kolmogorov-Smirnov test. In this study, linear regression allowed specifying to what extent predictor variables (such as demographic factors, clinical characteristics, or biomarker levels) are associated with depression and anxiety outcomes in SLE patients over a one-year period. For dichotomous variables, we used binary logistic regression to search for possible correlations between analyzed variables. To better understand the role of lupus-associated antibodies in relation to anxiety, depression, and quality of life, based on regression analysis results, we performed the Mann-Whitney test to compare groups positive for LA, anti-RIB P antibodies, anti-dsDNA, and anti-SM antibodies. We also identified differences regarding PAI-1, hsCRP, C4 levels, age, and disease duration using two-way ANOVA.

For multiple regression analysis, a stepwise procedure was implemented. To determine the validity of the WHODAS tool in detecting functional disability among SLE patients, we used exploratory factor analysis (EFA) and confirmatory factor analysis (CFA). To evaluate the effects of different types of medication on anxiety and depression scores in SLE patients, we used ANCOVA and the Kruskal-Wallis H test.

RESULTS

INITIAL RESULTS

After carefully reviewing the inclusion and exclusion criteria and obtaining informed consent, we included 65 adult patients diagnosed with SLE, from June 2019 to January 2020. Among the included patients, 5 (7.69%) were men and 60 (92.31%) were women. The average age of the study group was 51.48±13.85 years, and the average duration of lupus at the time of inclusion was 12.55±8.10 years. All included patients were on background therapy, in accordance with inclusion criterion 4. Thus, among the included patients, 35 (53.85%) were on oral corticosteroid therapy, of which 33 (50.76%) were on corticosteroid therapy associated with another background medication, adhering to a stable dose duration and excluding patients with doses over 20 mg/day due to the well-known potential to exacerbate neuropsychiatric symptoms (523). Among those on corticosteroid therapy, 2 (3.08%) were only on oral corticosteroid therapy as background treatment. Thirty-two (49.23%) patients were included without corticosteroid treatment. The average dose of corticosteroids used was 6.21 mg/day.

The most common background treatment was Hydroxychloroquine, with 61 (93.84%) of the included patients using this medication. Among these, 54 (84.61%) were on this treatment alone, 3 (4.62%) combined it with azathioprine 100 mg/day, 2 (3.08%) with mycophenolate mofetil 2 g/day, and 2 (3.08%) with methotrexate 10 mg/week. Among the patients on Hydroxychloroquine, 29 (53.70%) also combined it with chronic corticosteroid therapy. One patient (1.54%) was only on methotrexate 10 mg/week, and one (1.54%) on azathioprine 100 mg/day.

Initial Results: Depression, Anxiety, Quality of Life, Disability

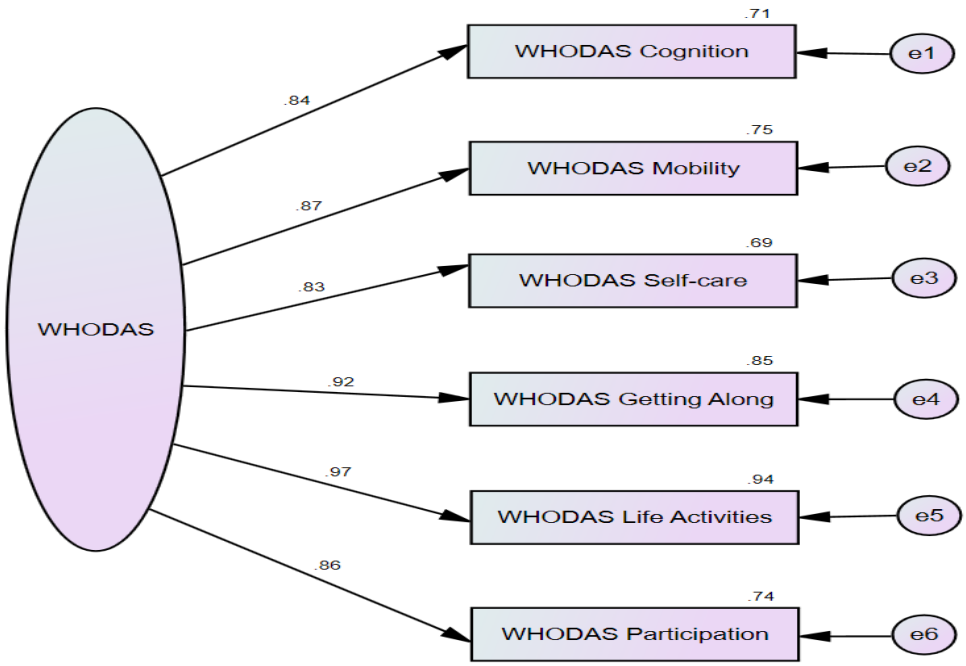
After analyzing the results, it was found that depression was present in 56 (86.15%) patients. Eight (12.31%) patients had severe depression, 22 (33.85%) had moderate symptoms, and 26 (40%) had mild depression.

The prevalence of anxiety in the study group was higher, with 64 (98.46%) patients experiencing anxiety. Among these, 16 (24.62%) patients reported very severe anxiety, 6 (9.23%) severe anxiety, 10 (15.38%) moderate anxiety, and 32 (49.23%) mild anxiety.

Evaluating disability using WHODAS 2.0, the results indicated a degree of disability in 52 (80%) patients, 6 (9.23%) had moderate disability, and 46 (70.77%) reported mild disability. The average disability score calculated using WHODAS 2.0 was 32.54%, corresponding to a moderate degree of disability, with a minimum of 1.88 and a maximum of 70.56%. The most affected domains, with moderate impairment, were socialization activities (average 45.77%), daily activities (45.53%), interpersonal relationships (average 33.38%), and mobility (33.10%). A mild degree of disability was reported for cognition (average 21.33%) and self-care (15.96%).

There was a strong correlation between the presence of depression and the degree of disability reported with WHODAS, the same for the presence of anxiety and disability, but a strong correlation between the presence of anxiety and depression was also noted.
The results of the linear regression analysis highlighted two models where anxiety (HAM-A) and disability reported by the WHODAS scale influence the level of depression (HAM-D). Regarding anxiety (HAM-A), the linear regression results indicate that depression (HAM-D) exerts a negative influence on anxiety, along with education level (an increase in depression severity is observed with lower education levels) and in combination with the total WHODAS score.
To evaluate the validity of the WHODAS instrument in detecting functional disabilities in SLE patients in our research, we conducted exploratory factor analysis (EFA) and confirmatory factor analysis (CFA). We applied Principal Axis Factoring as the extraction method and Varimax rotation with Kaiser Normalization. We checked the data regarding the assumptions of linearity and correlation, considering that all variables should have at least one correlation of $r \geq 0.3$. For assessing sample adequacy, we used the Kaiser-Meyer-Olkin (KMO) indicators and Bartlett's test of sphericity, considering KMO values > 0.5 and Bartlett $p < 0.05$ as adequate for EFA.
After EFA, we performed confirmatory factor analysis (CFA) using structural equation modeling (SEM). The parameters evaluated for model adequacy were: Root Mean Square Residual (RMR) below 0.08, Goodness-of-Fit Index (GFI) of at least 0.95, and Adjusted Goodness-of-Fit Index (AGFI) of at least 0.95. To identify internal consistency, we used Cronbach's alpha value.
Figure number 8 illustrates schematically the results of CFA. Regarding reference indicators, we have RMR = 0.078, GFI = 0.997, and AGFI = 0.994, and the minimum model fit was achieved.

Figure 8: CFA Diagram in Structural Equation Modeling (SEM) for Evaluating the WHODAS Scale.



Thus, according to the results, there is an extremely strong positive correlation between the level of depression and overall functional disability, as assessed with the WHODAS 2.0 instrument. These

findings suggest that WHODAS could represent a pertinent tool for evaluating disability associated with depression and anxiety symptoms in SLE patients.

Clinical and Biological evaluation for SLE- initial results

At the time of inclusion, the specific lesions of SLE in the patient group were as follows: musculoskeletal involvement present in all 65 (100%) patients, cutaneous-mucosal lesions in 53 (80%) patients, serosal involvement in 46 (70.77%) patients, renal involvement in 18 (27.69%) patients, cardiac involvement in 17 (26.15%) patients, neurological/neuropsychiatric involvement in 35 (53.85%) patients, and hematological involvement in 59 (90.77%) patients.

Patients were evaluated at inclusion for irreversible lesions based on the SLICC ACR score. Thus, 17 (26.15%) patients had no irreversible lesions at inclusion, most patients had at least one irreversible lesion, cumulating a score of 1 in 26 (40%) patients, a score of 2 in 12 (18.46%) patients, a score of 3 in 5 (7.69%) patients, and scores of 4 and 5 in one patient each (1.54%), a score of 6 in 2 (3.08%) patients, and one patient with the maximum irreversible lesion score of 8.

Antinuclear antibodies were present in 52 (80%) of the patients, statistically significant at $p<0.0001$, with a minimum considered positive titer of 1/80 and a maximum of 1/1280. Thirteen (20%) of the patients had titers below 1/80. The ANA typing results were as follows: anti-dsDNA antibodies specific to SLE were present in 42 (64.4%) of the patients, statistically significant at $p<0.05$, with an average value of 50.76 U/ml and a maximum of 468 U/ml, while 23 (34.5%) of the patients had absent anti-dsDNA antibodies. Only 2 (3.1%) patients had positive anti-Sm antibodies. Anti-Ro antibodies were positive in 33 (50.8%) patients. Anti-ribosomal P protein antibodies associated with NPSLE pathology were detected in 28 (43.1%) of the patients, with an average value of 26.64 U/ml but with maximum values exceeding 200 U/ml, a percentage at the upper limit compared to literature data (10-40%) (528).

The reduction of complement fractions, an important marker of disease and activity, was present as follows: for the C3 fraction in 48 (73.8%) of the patients, statistically significant at $p<0.0001$, with an average value of 84.51 mg/dl, below the lower limit value of 90 mg/dl, and C4 in 17 (26.2%) patients, with an average of 16.04 mg/dl.

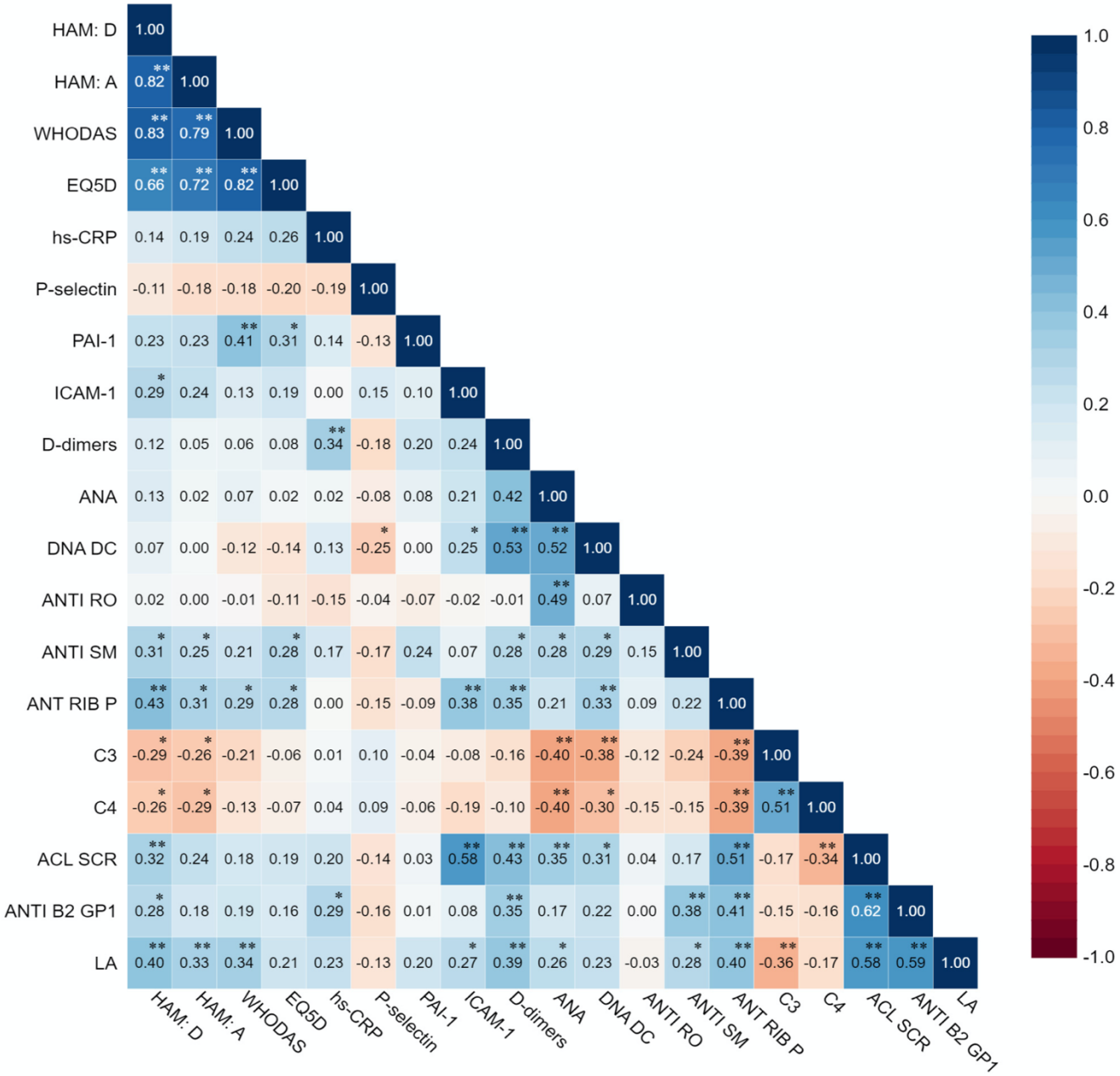
Antibodies associated with antiphospholipid syndrome were present as follows: screening anticardiolipin antibodies in 36 (55.4%) patients, with an average value of 26.79 U/ml, and 29 (44.6%) had negative values. Anti-beta 2 glycoprotein I antibodies were present in 31 (47.7%) patients, with an average value of 23.90 U/ml, absent in 34 (52.3%) patients, with the presence of lupus anticoagulant reported in an identical number of patients, respectively, 31 (47.7%) patients, absent in 34 (52.3%) patients.

In our study group, no patient had positive values for PAI-1, and 1 (1.5%) patient had a positive value for P-selectin. D-dimers were positive in 10 (15.4%) patients, and 20 (30.8%) patients had positive values for ICAM-1, with an average value of 97.73 U/ml, while the remaining 45 (69.2%) patients had negative values.

Correlations made between biomarkers associated with thrombosis and inflammation, and the presence of depression, anxiety, quality of life as measured by EQ-5D, and the degree of disability as reported by WHODAS 2.0, allowed the creation of a heat map that vividly reflects the importance of existing relationships (fig.nr.9).

An extremely significant correlation was noted between depression and the presence of anticardiolipin antibodies, lupus anticoagulant, and anti-ribosomal P antibodies. Strong correlations were also identified with ICAM-1, low C4 fraction levels, and the presence of anti-Sm antibodies.
Anxiety showed a close correlation with the presence of lupus anticoagulant, as well as with anti-ribosomal P antibodies, low C3 and C4 fraction levels, and the presence of anti-Sm antibodies.
Significant values for ICAM-1 were correlated with the presence of anti-dsDNA antibodies, ACL antibodies, and the presence of lupus anticoagulant. Anti-ribosomal P antibodies showed correlations with depression and anxiety within our study group, as well as with disability evaluated with WHODAS. Biologically, they correlated with the presence of ICAM-1, D-dimers, homocysteine, and anti-dsDNA antibodies. They were also strongly correlated with low C3 and C4 fraction levels, ACL antibodies, anti-beta 2 GP1, and lupus anticoagulant. Quality of life, assessed with EQ-5D, showed correlations with the presence of PAI-1, anti-ribosomal P antibodies, and anti-Sm antibodies (fig.nr.9).

Fig.nr.9 Heat Map of Correlation of Biomarkers Associated with Thrombotic/Inflammatory Pathways and Depression, Anxiety, Disability, Quality of Life in SLE Subjects



(p < 0,01, * p < 0,05)**

In another statistical analysis model, linear regression, targeting the association between the presence of depression, anxiety, the degree of disability reported by WHODAS, and quality of life reflected by EQ-5D in relation to the studied biomarkers, reveals a statistically significant association of depression reported by HAM-D with the presence of anti-ribosomal P protein antibodies, absence of PAI-1, and anxiety with the presence of LA and low values of the C4 complement fraction. The degree of disability reported by WHODAS was significantly correlated with anti-ribosomal P protein antibodies, which appears to be the most relevant biomarker for this study, also related to the quality of life reported by EQ-5D, both inversely correlated with the titer of anti-dsDNA antibodies (Table no.22).

Table no.22. Linear Regression Results of the Association in SLE between Depression, Anxiety, Quality of Life, and Biomarkers.

Parameter	Association/R^2	B (CI)	*p*
HAM D*	Ab anti-ribozome P/ 0.183	0.070 (0.33-010)	<0.001
	PAI 1/0.258	2.949 (0.60-5.30)	0.014
HAM A	LA/0.109	0.485 (0.13-0.83)	0.007
	C4/0.166	-0.026(-0.05-0.001)	0.043
WHODAS	PAI 1/0.169	8.965 (3.97-13.96)	0.001
	Ab anti-ribozome P / 0.274	0.109 (0.04-0.18)	0.004
	DNA DC/0.330	-0.052(-0.10-0.01)	0.028
	CRP/0.377	16.356(1.07-31.64)	0.036
EQD5	PAI 1/0.096	0.164 (0.04-0.29)	0.012
	Ab anti-ribozome P / 0.192	0.003 (0-0)	0.009
	Ab anti dsDNA/0.256	-0.001 (0-0)	0.025
	CRP/0.320	0.460 (0.07-0.85)	0.021

To determine the impact that the main studied biomarkers may have on depression, anxiety, and disability in SLE patients, the nonparametric test conducted indicates that the presence of anti-ribosomal P protein antibodies, lupus anticoagulant, anticardiolipin antibodies, and anti-beta 2 GPI significantly increases the risk of developing depression and anxiety disorders compared to the group of patients with negative biomarkers, becoming practically the most important predictors according to the current research. The degree of disability does not seem to be correlated with these biomarkers, but the quality of life reported by EQ-5D may vary depending on the presence of anti-ribosomal P protein antibodies and LA. The initial evaluation results indicate a high prevalence of depression and

anxiety in the study group of SLE patients, supporting active screening for the studied disorders. Among the evaluated serum biomarkers, the presence of anticardiolipin antibodies and anti-beta 2 glycoprotein I, lupus anticoagulant, ICAM-1, low C4 complement fraction, and anti-ribosomal P antibodies represent risk factors, suggesting that both the autoimmune/inflammatory pathway and the ischemic/thrombotic pathway could contribute to these manifestations of NPSLE.

Psychiatric Treatment in the Study Group

Following the initial evaluation, which revealed elevated levels of depression and anxiety scores, each patient who presented changes in depression and/or anxiety scores was evaluated by a psychiatric specialist who provided personalized treatment recommendations according to current local guidelines. Consequently, 34 (52.31%) subjects did not have an indication for treatment, 4 (6.15%) subjects received only anxiolytic treatment, 7 (10.77%) patients were given antidepressant therapy, while 20 (30.77%) subjects received both antidepressant and anxiolytic medications. The classes of drugs used were selective serotonin reuptake inhibitors (SSRIs): Fluoxetine 20mg/day, Escitalopram-Cipralex 10mg/day, Paroxetine-Seroxat 20mg/day; selective serotonin and norepinephrine reuptake inhibitors (SNRIs): Venlafaxine 75mg/day, Duloxetine 60mg/day; other antidepressants - Trazodone (Triticco) 150mg/day; tricyclic antidepressants - Amitriptyline 25mg/day; benzodiazepines: Alprazolam (Xanax) 0.5-1.5mg/day, Lorazepam (Anxiar) 1mg/day.

FINAL EVALUATION RESULTS AT 12 MONTHS

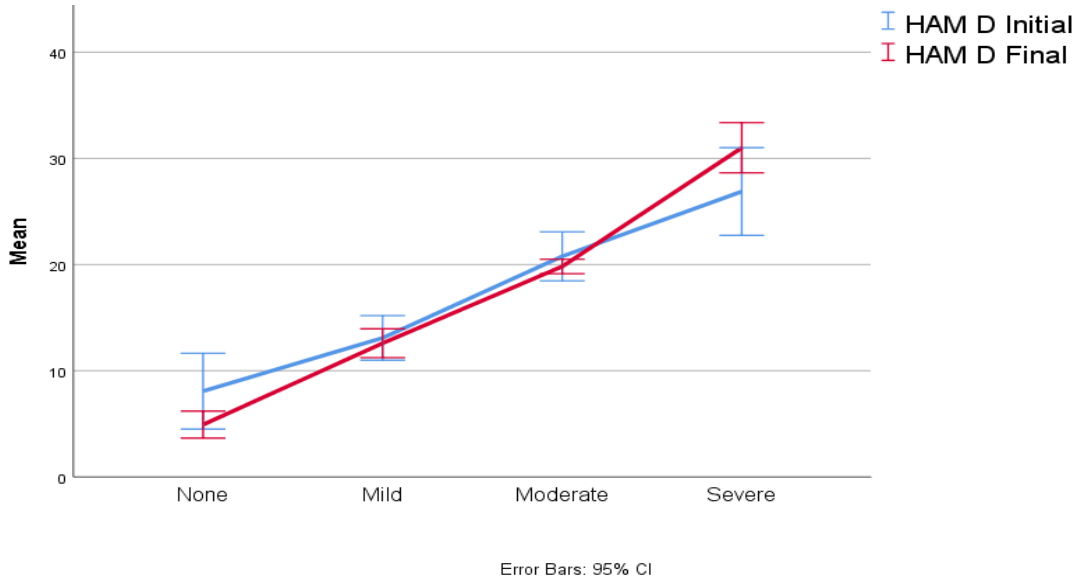
All 65 patients were re-examined according to protocol at 12 months. Cardiovascular risk factors, background and concurrent therapy, complete physical examination, vital signs, HAM-D, HAM-A scales, EQ-5D questionnaires, Pain VAS, C-SSRS, WHODAS 2.0, and paraclinical assessments were reevaluated through a complete immunological profile associated with SLE, complement fractions, CBC, biochemistry, proteinuria, with recalculation of activity and irreversible lesion scores: SLEDAI-2K, BILAG 2004, SFI, and SLICC-ACR. The results obtained after one year are presented below.

Evolution of Depression and Anxiety

The results obtained from the HAM-D depression scale evaluation at one year reassessment,indicate a persistently high prevalence of depression, with 52 (80%) patients reporting some degree of depression. A slight decrease is observed compared to the initial evaluation, where it was detected in 56 (86.15%). Regarding the severity of manifestations, they remain persistently severe in 8 (12.31%) patients compared to the initial evaluation, similarly for moderate symptoms reported by 22 (33.85%) patients, while mild symptoms tend to decrease slightly from 26 (40%) at the initial evaluation to 22 (33.85%) at the one-year evaluation. The average depression score obtained with the HAM-D scale at the initial visit was 16.385 (standard error [SE] r-0.989, 95% CI lower limit 14.408-upper limit 18.261), and at the 12-month reevaluation, the average was 15.769 (SE r-1.019, 95% CI lower limit 13.733-upper limit 17.806), without statistically significant differences (p-0.311) according to paired comparison or multivariate tests.

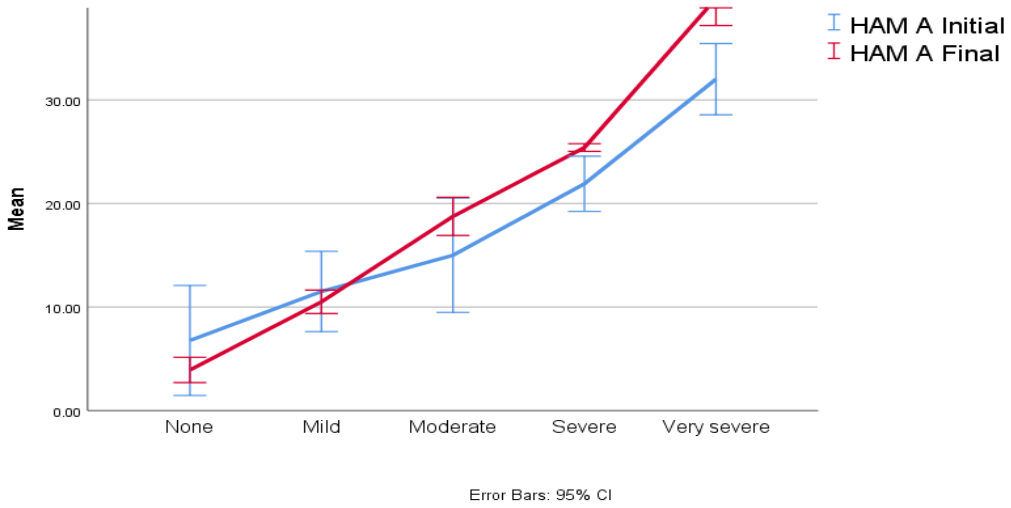
Only 3 patients who initially reported depressive symptoms had a favorable evolution with symptom resolution at 12 months. A relevant graphical representation of the evolution of depression at 12 months compared to the initial moment is presented below (Graph no.37).

Graph no. 37 The evolution of depression at the one-year reassessment



Similarly to the evolution of depression, at 12 months, persistently high levels of anxiety are observed, reported by an identical number of patients as in the initial evaluation, specifically 64 (98.46%). Unfortunately, besides the consistently high prevalence, an increase in severity was also noted. Thus, the number of patients with very severe anxiety showed an upward trend from 16 (24.62%) at the beginning to 22 (33.85%) at the 12-month evaluation. The same increasing trend was observed among patients with severe anxiety, from 6 (9.23%) at the beginning to 9 (13.85%) at 12 months, and for those with moderate anxiety, from 10 (15.38%) to 12 (32.3%) at 12 months. Unlike the evolution of depression, where the mean score at 12 months remained similar without significant statistical changes, the reporting of anxiety using HAM-A shows an increase in the mean from 19.1077 initially (standard deviation 12.07933) to 22.0154 (standard deviation 14.20221). The results of the variance analysis (ANOVA) indicated a statistically significant difference, with p=0.006, F (1,64)=8.049, and a confidence interval (CI) ranging from 0.086 to 4.96. The average anxiety score showed an increase of 2.91 (2.12) points, as presented in graph no.39.

Graph no.39: The evolution of anxiety at the one-year reassessment



To identify possible factors that could underlie the evolution of depression and anxiety at 12 months, we included in the statistical analysis the parameters obtained at both the initial and final evaluations, performing linear regression tests, correlations, and ANCOVA analysis, obtaining the following results. Thus, linear regression established a crucial relationship, namely that the progression of anxiety at 12 months is significantly influenced by the initial level of depression (R2=0.536). These results reiterate the critical importance of timely recognition and treatment of mood disorders in individuals with SLE to improve quality of life and additionally prevent the onset of new and severe conditions.
The evolution of lupus disease at 12 months was analyzed not only in terms of disease activity but also new irreversible lesions using the SLICC/ACR scale. Regarding the possible pathogenesis that could influence the evolution of depression and anxiety at 12 months in the study group patients, we revisited the correlations with potential biomarkers considered to be involved.
At the initial evaluation, nonparametric tests performed for HAM-A, HAM-D indicated that in the group of SLE patients, those positive for biomarkers such as anti-ribosomal P protein antibodies, LA, anticardiolipin antibodies, or anti-beta 2 glycoprotein I are at higher risk of developing depression and anxiety compared to patients with negative values for these biomarkers (529). All biomarkers initially evaluated (hsCRP, P-selectin, PAI-1, ICAM-1, D-dimers, homocysteine, ANA, anti-dsDNA antibodies, anti-Ro antibodies, anti-Sm antibodies, anti-ribosomal P protein antibodies, C3, C4, ACL-SCR, anti-beta 2-GP1 antibodies, LA) were included in correlation and regression tests. Alongside the results obtained at 12 months on the HAM-D, HAM-A scales, only statistically significant results are included in Table no.32, thus those biomarkers that can impact the 12-month evolution of mood disorders.

Table no.32. Specific Biomarkers Predictive of NPSLE Manifestations at One Year

Scale	Biomarker	R2	B	p
Initial HAM A	LA C4	0.108 0.165	6.78 (1.88-11.67) -0.37 (-0.72-0.01)	**0.007** **0.044**
Final HAM A	LA Anti RIB P/	0.149 0.096	9.40 (3.71-14.97) 0.091 (0.02-0.16)	**0.002** **0.012**
HAM A evolution(Final-Initial)	C4 D-dimers	0.156 0.234	0.41 (0.17-0.65) 0.01 (0-0.02)	**0.001** **0.015**
Initial HAM D	Anti RIB P/ PAI	0.183 0.258	0.070 (0.33–010) 2.949 (0.60–5.30)	**<0.001** **0.014**
Final HAM D	Anti Rib P LA	0.336 0.396	0.098 (0.06-0.13) 3.755 (0.74-6.77)	**<0.001** **0.015**
HAM D evolution(Final-Initial)	D-dimers	0.104	0.008 (0.0-0.001)	**0.009**

At 12 months, the depression score,correlates once more with the presence of anti-ribosomal P protein antibodies and LA, while the variability of the final score compared to the initial one is related to the D-Dimer value. The same predictors for anxiety levels at 12 months: the presence of anti-ribosomal P protein antibodies and LA, while the variability of the score can be linked to low C4 levels and D-Dimers.
The presence of specific biomarkers, such as LA and anti-Ribosomal P antibodies, appears to be associated with the persistence of both depression and anxiety, indicating a dual and complex underlying pathogenic mechanism involving inflammation and thrombosis.
By accumulating initial data and observing at 12 months, it can be seen that the strongest predictors of depression and anxiety in SLE patients are LA and anti-ribosomal P protein antibodies, demonstrating once again that the pathogenesis of these disorders is dual and incompletely elucidated.

Evolution of SLE disease at 12 Months

Patients were monitored clinically and biologically as mentioned, according to the work protocol, with activity elements included in the utilized scales SLEDAI-2K, BILAG, and SFI.
At 12 months, the SLEDAI-2K score indicates no activity with a score of 0 in 8 (12.3%) patients, mild activity with a score of 1-5 in 31 (47.69%) patients, moderate activity with a score of 6-10 in 24 (36.92%) patients, and high activity with a SLEDAI score of 11-19 in 2 (3.1%) patients. No patient had a score over 20 corresponding to very high activity. Thus, it can be noted that 26 (40%) patients had a lupus activity flare based on SLEDAI-2K criteria, with an increase of over 6 points compared to the initial evaluation, despite consistently following background therapy for SLE disease.
Based on the increase in the SLEDAI-2K score, the intention to treat the activity flare, and the increase in VAS, flares were evaluated according to SELENA SLEDAI Flare Index (SFI) criteria. Thus, at the 12-month evaluation, 29 (44.62%) patients had a moderate SFI flare, one patient (1.54%) had 2 moderate flares, and 6 (9.23%) patients had a severe SFI flare. Concluding, 36 (55.39%) patients had a flare, while 29 (44.61%) did not have a flare requiring treatment.
Regarding disease activity according to the BILAG-2004 scale, the results also indicate the occurrence of flares, with 5 (7.7%) patients meeting criteria for BILAG-A, 17 (26.15%) patients having criteria for 2 BILAG-B, both situations classifiable as disease flares, and 20 (30.77%) patients having criteria for only one BILAG-B. Thus, according to BILAG-2004 criteria, 22 (33.84%) patients presented with a flare requiring therapeutic intervention. The evolution of SLE disease at 12 months was analyzed not only from the perspective of activity but also regarding the appearance of new irreversible lesions using the SLICC/ACR scale.
Compared to the initial evaluation when 17 (26.15%) patients had no irreversible lesions, currently, 2 more such lesions were acquired. It can also be observed an aggravation of lesions, so compared to the initial evaluation when 26 (40%) had one organ lesion, the number decreased to 22 (33.85%), with an increase in the number of patients with higher scores, respectively 2 to 16 (24.62%) patients compared to 12 (18.46%) initially, one additional patient with a score of 3, and 4, and an increase from a score of 6 to 7, thus not only an increase in the number of patients with irreversible lesions but also in the severity of the lesions.

Serum Biomarker Interrelation and Lupus Disease Evolution at 12 Months

The increased activity evolution of SLE disease at the 12-month reevaluation, revealed by BILAG, SLEDAI, and SFI, required a careful investigation of possible impacting factors influencing this disease evolution pattern. Thus, statistical analysis approached multiple models using correlation and regression tests with the dependent variables mentioned above.

Regarding the disease evolution evaluated by SLEDAI-2K, the Pearson correlation test revealed a weak relationship with hsCRP, difficult to evaluate considering this biomarker's normal values in the studied group. In linear regression, the SLEDAI score strongly correlates with anti-ribosomal P protein antibodies, ANA, presence of LA, and P-selectin.

Pearson correlation results indicate a statistically significant association between the BILAG-A score and ANA presence ($p=0.006$), consistent with linear regression ANOVA results, where the only constant, statistically significant predictor was also the presence of ANA.

Pearson correlation results show that the BILAG-B score has a statistically significant relationship with hsCRP, PAI-1, ICAM-1, anti-Sm antibodies, and C3 fraction, and linear regression ANOVA identified constant predictors as PAI-1, highly significant, and C3, ICAM-1, hsCRP.

Disease activity reflected by a moderate SFI flare presents significant correlation (Pearsons) with C3 and PAI-1, relationships reconfirmed by the ANOVA test where constant predictors are PAI-1 and C3.

Severe flare according to SFI showed significant correlation in the Pearson test with ANA presence, anti-dsDNA antibodies, and anti-Sm antibodies, C3, and ANOVA regression test indicated constant predictors as ANA and anti-Sm antibodies.

Regarding irreversible lesions evaluated by SLICC/ACR, Pearson correlation test did not reveal significant results, in the ANOVA regression model, predictable results outlined the presence of ANA and anti-dsDNA antibodies.

Evolution of SLE Disease in Correlation with Depression and Anxiety

The initial presence of depression can significantly statistically influence the 12-month evolution of disease activity reflected by BILAG with severity criteria A (moderate correlation) and B (high correlation), SLEDAI-2K, moderate activity flare at SFI (moderate correlation). Anxiety impacts the activity score reported by BILAG-B (strong correlation), SLEDAI-2K (moderate correlation), SFI-moderate (high correlation), similarly to the degree of disability initially reported by WHODAS 2.0. Severe activity flare or irreversible lesions were not correlated with initial values reported on HAM-D, HAM-A, WHODAS.

A more complex analysis of SLE activity evolution, based on the linear regression model, included the mentioned biomarkers and the initial values of depression and anxiety, the degree of disability reported by WHODAS 2.0, background medication suggested that lupus disease activity increases with disability (WHODAS) and anxiety (HAM A).

The evolution of SLE reflected by moderate disease activity at SFI was influenced at one year by the WHODAS score ($p<0.0001$), while severe SFI activity was influenced by the initial dose of background corticosteroid therapy.

Response to Psychiatric Therapy

The previously presented results ,showing high levels of depression and anxiety ,required therapy according to recommendations, and the impact of psychiatric treatment recommendations on the one-year evolution of depression and anxiety was carefully analyzed statistically.
We studied the effect on the evolution of HAM-D and HAM-A scores by classes of anxiolytic and antidepressant medications and their combinations.
Pairwise analysis of anxiolytic medication allowed identifying the drug and dose that has a statistically significant impact on depression evolution, thus indicating that it is statistically significant for the patient to receive treatment at a minimum dose of anxiolytic, both Alprazolam (Xanax) 0.5mg/day and Lorazepam (Anxiar) 1mg/day without differences at higher doses. The analysis highlights that low-dose anxiolytic therapy has a favorable impact on depressive symptoms, an important element to consider when choosing psychiatric therapy in SLE patients, as clearly shown in the graphical representation.
We also studied the impact of antidepressant therapy on anxiety scores reported at 12 months, with pairwise analysis indicating the major importance of initiating therapy versus the lack of antidepressant therapy that can impact anxious manifestations in SLE patients, with benefits even at a minimum dose without significant differences at higher doses or the type of antidepressant used. Antidepressant treatment with Venlafaxine, Paroxetine (Seroxat), Fluoxetine (Prozac) does not bring therapeutic benefits regardless of dose and there are no significant differences between different drugs and doses.
The evaluation of the therapeutic efficacy of antidepressant medication on depression evolution did not reveal significant results in either pairwise analysis or the Kruskal-Wallis test. However, the one-year evolution of depression appears to be significantly impacted by anxiolytic treatment and its combination with antidepressant treatment.
In pairwise comparison of medication, combined antidepressant and anxiolytic therapy is the only one with statistically significant efficacy and apparently, antidepressant therapy may only work in combination with anxiolytic therapy, even at low doses as previously demonstrated, surprising results with possible impact on therapeutic recommendations in SLE patients.
The statistical analysis of the therapeutic impact on the one-year evolution of depression and anxiety was completed with ANCOVA covariance analysis, which included psychiatric medication and background lupus disease therapy. No statistically significant influence of antidepressant therapy on the evolution of anxiety or depression was found. However, it appears that combining Hydroxychloroquine (Plaquenil) with Methotrexate may have a significant impact on the evolution of affective disorders.

Table no.47 Results of the ANCOVA Test on the Impact of Therapy on the Evolution of Depression and Anxiety in SLE Patients

Group and therapy	Therapeutic group(N)	Estimated mean (CI)	R2	P
Depression and psychiatric therapy	Anxiolytic (24) None (41)	13.65 (11.46-15.85) 17.01 (15.42-18.59)	0.698	0.024
	Anxiolytic + antidepressant (20) Antidepressant (7)	14.13 (11.51-16.74) 21.18 (17.48-24-87)	0.734	0.003
	Azathioprina+ Hidroxicloroquina (3) Methotrexate+ Hidroxicloroquina (1)	20.89(15.80-25.98) 3.87(-4.96-12.69)	0.745	0.024
Depression and SLE medication	Azathioprina+ Hidroxicloroquina (3) Methotrexate+ Hidroxicloroquina (1)	20.89 (15.80-25.98) 3.87 (4.96-12.69)	0.075	0.030
Anxiety and SLE medication	None(3) Methotrexate+ Hidroxicloroquina (1)	30.44 (22.74-38.15) -9.77 (6-23.29-3.75)	0.804	<0.001
	Azathioprine (1) Methotrexate+ Hidroxicloroquina (1)	23.20 (9.76-36.64) -9.77 (6-23.29-3.75)		0.019
	Methotrexate (1) Mycophenolate + Hidroxicloroquina(2)	45.10 (31.75 -58.45) 16.00 (6.37-25.62)		0.017
	Methotrexate (1) Hidroxicloroquina (54)	45.10 (31.75 -58.45) 21.92 (20.11-23.74)		0.023
	Hidroxicloroquina (54) Methotrexate+ Hidroxicloroquina (1)	45.10 (31.75 -58.45) 16.00 (6.37-25.62)		<0.001
	Azathioprine+ Hidroxicloroquin (3) Methotrexate+ Hidroxicloroquin (1)	21.75 (14.03 -29.47) -9.77 (6-23.29-3.75)		0.03

Therefore, regarding the impact of different types of medications on depression and anxiety manifestations after one year of follow-up, the results suggest that various combinations of medications for SLE, such as Methotrexate and Hydroxychloroquine, can significantly reduce levels of anxiety and depression compared to other pharmacological therapies. Additionally, the level of depression may be influenced by anxiolytic therapy. ANCOVA and linear regression results do not suggest any influence or prediction regarding neuropsychiatric therapy on anxiety symptoms in SLE.

DISCUSSIONS

Discussion of Initial Results

The study group included 65 patients, and an initial discussion could be related to the size of the evaluated group. However, this is a pathology with low prevalence in the general population, conducted monocentrically. Moreover, most observational studies on SLE in the literature conducted in European countries include between 60-100 patients, as evidenced by the results of meta-analyses, such as the one recently published in 2023. (530)

In our research, based on evaluations using the HAM-D17 and HAM-A scales, we found high rates of depression and anxiety in SLE patients, namely 86.15% and 98.46%, respectively. These figures are significantly higher than those reported in previous studies, where similar research indicated a rate of 45.2% for depression and 37.1% for anxiety.(439)

A more recent meta-analysis published in 2020 shows a wide variability in the reported prevalence of both depression (2.1-78.6%) and anxiety (2.9-84.9%) in SLE patients, obviously related to the scales used, study quality, and different definitions.(51,424)

In our study group patients with SLE tended to have a higher prevalence of anxiety than depression, consistent with previous studies.(548,436) Additionally, 48.38% of patients reported both depressive and anxiety symptoms, similar to other studies.(439)

The evaluation of disability degree using the WHODAS 2.0 scale indicated a moderate level of 32.54%, with similar moderate to severe disability data reported in a fibromyalgia study, with an average of 43.8 ± 16.5, also correlating with the presence of anxiety. (555)

Unfortunately, published data related to the results of applying WHODAS in SLE patients are limited. The use of the scale in this study allowed for the evaluation of WHODAS validation in SLE patients through statistical exploratory and confirmatory factor analyses, suggesting that this scale could be an important tool for evaluating disability in SLE patients, as already published**. (556)**

An important part of our study was related to the evaluation of biomarkers in lupus disease, bringing originality to the work as we evaluated serological markers associated with both inflammation and coagulopathy to explore as closely as possible the dual pathogenic mechanism potentially associated with NPSLE. **(556)**Among the antibodies associated with neurological determinations in SLE, anti-ribosomal P protein antibodies were detected in 43.1%, a higher percentage compared to previous studies, especially those reported in Europe (570) but closer to those reported in the Asian population with a percentage of 42.9%.(571)

Antiphospholipid syndrome-associated serology was also detected in a significant percentage: anti-cardiolipin antibodies in 55.4% of patients, anti-beta 2 glycoprotein I antibodies in 47.7% of patients, identical to the presence of LA, slightly higher than the literature citing a positivity rate of 30-40%, LA present between 11-30%, and anti-cardiolipin antibodies 17-40%, (573,244,574) but the clinically significant prevalence is approximately 20%,(575) linked to LA positivity but also moderate/high titer of ACL or anti-beta2GPI (≥40 U or ≥99th percentile), compared to low titer, IgG, IgM compared to IgA(576) or triple positivity.(577) Comparison in our study group between subjects with positive and negative ACL suggests that anxiety and depression are significantly higher in SLE patients with positive ACL. Our research results indicate that coagulation-associated biomarkers, along with specific inflammation markers, are correlated with depression and anxiety in SLE patients. A strong positive correlation has been demonstrated between depression, anxiety, and anti-cardiolipin and anti-beta 2 glycoprotein I antibodies, lupus anticoagulant, ICAM-1, low C4 values, and anti-ribosomal P protein antibodies. Disability evaluated with WHODAS 2.0 and quality of life evaluated with EQ-5D seem to be influenced by high levels of PAI-1, anti-ribosomal P, hsCRP, and low levels of anti-dsDNA antibodies.**(529)**

Discussion of reassessment results at one year

Depression levels remained high at one year in 80% of patients, with a similar severity degree as initially, while anxiety prevalence remained high at 98.46%, with an increase in severity—surprising results for which we tried to find significant explanations and correlations. A possible explanation for this aspect could be that the one-year re-evaluation of patients occurred in the context of the COVID-19 pandemic, with literature data supporting the impact that the pandemic had on the worsening of affective disorders. (584,585)

In a study conducted by Mak and colleagues in 2011, it was observed that anxiety in SLE patients could be anticipated based on factors such as the presence of depression, a high cumulative dose of glucocorticoids, and the use of other medications. Moreover, the intensity of depression appeared as a predictor for the severity and existence of anxiety. Therefore, individuals with lupus who exhibit anxiety symptoms should be subjected to a concomitant and detailed evaluation for potential coexisting depression. (548) Similarly, our study established through linear regression that the progression of anxiety at 12 months is influenced by the initial level of depression and that the level of anxiety increases in relation to the level of depression.

Despite the high prevalence, few data can be found in the literature on the long-term evolution of depressive or anxiety symptoms in SLE patients. This study has an original contribution through its longitudinal design with 12-month monitoring. A study that followed the evolution of depression in SLE patients for 48 months showed that depression in SLE patients is persistent despite therapeutic interventions for pain, affective disorder, and lupus background treatment, similar to the results obtained in the present research.(591)

In a study that monitored cognitive dysfunction in SLE patients for 5 years but also tracked depressive symptoms, the same persistent trajectory of these symptoms was indicated. (592) Compared to biomarkers initially associated with the presence of depression and anxiety, already published data**(529)** suggest that anti-ribosomal P protein antibodies and LA remain risk factors for the persistence of depression and anxiety at 12 months, again suggesting that the pathogenesis of these disorders in SLE is complex and multifactorial, involving inflammation and coagulation.

Regarding psychiatric therapy, literature data indicate selective serotonin reuptake inhibitors (SSRIs) as the first-line treatment for affective disorders due to their safety and tolerability profile(609), but their use remains empirical in the absence of controlled studies.(610)

In our study, we used escitalopram, fluoxetine, and paroxetine, reported in other studies as effective in depression associated with SLE. (611,612,613,614) For anxiety, antidepressants such as SSRIs and anxiolytics such as benzodiazepines can be prescribed according to standard indications in primary psychiatric disorders, recommendations followed in our study but, like depression treatment, remain empirical in the absence of studies. (610,615)

In our study, neuropsychiatric treatment had no influence on the evolution of anxiety and depression, although reported data show increased efficacy of antidepressants at 62.1-70%,(616) while others have limited efficacy at 7%. (617)

An alternative explanation for the limited efficacy of prescribed selective serotonin/noradrenaline reuptake inhibitors (SSRI/SNRI) could be their influence on sleep quality; these drugs have been associated with impaired sleep quality, a common problem affecting at least half of people with SLE. Interestingly, possibly related to the impact that anxiolytics have on sleep, the anxiolytic treatment of our patients shows a favorable impact on depression. Similar data indicate that patients with depressive symptoms could benefit from adding anxiolytic treatment and should be considered as an addition to SSRI therapy, although short-term due to the increased risk of dependency and cognitive deterioration. (621,622,623)

As part of the background treatment for systemic lupus erythematosus , our data suggest that combining methotrexate and hydroxychloroquine may offer additional benefits by potentially addressing inflammation and thrombotic risk.(631)

CONCLUSIONS. STRENGTHS OF THE THESIS. NEW RESEARCH DIRECTIONS

Conclusions from Initial results

The initial results reveal a high prevalence of depression and anxiety in the group of SLE patients, an extremely important aspect that underlines the necessity of active screening for these symptoms and manifestations within this population. Among the serological biomarkers, there is a significant positive correlation between depression, anxiety, and various antibodies, such as those associated with cardiolipin, beta2-glycoprotein I, lupus anticoagulant, ICAM-1, low C4, and Anti RIB P antibodies. These findings indicate that both the autoimmune/inflammatory pathway and the ischemic/thrombotic pathway could contribute to depression and anxiety as manifestations of NPSLE. Therefore, it is important to develop specific serological markers for NPSLE to enable early diagnosis and treatment, and to revise treatment guidelines accordingly.

Conclusions from one year reassessment results

After 12 months of careful monitoring and personalized treatment, adapted for both SLE and NPSLE, the prevalence and severity of depression and anxiety remain significantly high in our SLE patients. The presence of specific biomarkers, such as LA and Anti-Ribosomal P antibodies, seems to predict the persistence of both depression and anxiety, further suggesting the involvement of a complex dual

inflammatory-thrombotic mechanism as an underlying pathogenic determinant. Moreover, the findings suggest that while anxiety levels tend to increase over time, they do not show a direct correlation with SLE activity.

The results indicate that levels of antinuclear antibodies and the PAI-1 biomarker can predict SLE disease activity at one-year follow-up. Specific medications for SLE, such as Methotrexate and Hydroxychloroquine, correlate with reduced anxiety and depression scores. Additionally, anxiolytic therapy seems to reduce depression but without impacting anxiety disorders, remaining an essential issue in NPSLE treatment. The relevant data presented indicate a high prevalence of depression and anxiety, along with long-term persistence and a significant impact on functioning, quality of life, and lupus disease progression. In conclusion, the study highlights the necessity for ongoing research and the development of more effective therapeutic interventions for NPSLE. Understanding the complex interaction between SLE and neuropsychiatric symptoms is crucial for improving patient outcomes. There is a clear need for more robust longitudinal studies to explore the multifaceted nature of SLE and its impact on mental health, aiming to improve the quality of life for those affected by this complex autoimmune disease. These studies should target the development of biomarkers and the creation of a comprehensive and complex evaluation algorithm, essential for current medical practice.

Strengths and Originality of the Thesis

The studies conducted have significantly contributed to detecting the prevalence of depression and anxiety in SLE patients in Romania and their functional impact, performing the first evaluation of the feasibility of the WHODAS 2.0 disability scale in SLE patients and the first statistical validation in this pathology. We have provided data related to the pathogenesis of SLE, focusing on the two prevalent inflammatory and thrombotic pathways, establishing potential serological biomarkers associated with NPSLE. The 12-month longitudinal study followed the evolution of anxiety-depressive symptoms as well as lupus disease in terms of activity, progression, and possible predictive factors of evolution. Additionally, it evaluated the therapeutic response to standard psychiatric therapies among SLE patients, data that have been scarcely published until now.

Future Research Directions

- Establishing a program for active detection and long-term monitoring of affective disorders in SLE patients with the implementation of early diagnostic and impactful therapeutic intervention strategies.
- Given the increasing prevalence of autoimmune pathologies, with potential physical and mental impact, procedures for screening anxiety, depression, and cognitive disorders among all patients with systemic autoimmune diseases can be developed.
- Broader research to elucidate the etiopathogenesis of neuropsychiatric manifestations in SLE to establish serological and other biomarkers that allow early diagnosis and targeted, personalized therapeutic intervention to treat, improve quality of life, and recover SLE patients.