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TESI DI LAUREA

Efficacy of belimumab on specific and nonspecific skin manifestations of systemic lupus erythematosus. Results from a multicentric, nationwide cohort of patients treated in a real-world setting (BeRLiSS-Skin).

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TABLE OF CONTENTS

ABSTRACT

Background: The efficacy of belimumab in SLE has been extensively proved, both in randomized controlled trials, and real-life observational studies. However, there are no studies in which patients were stratified by subtype of skin manifestations.

Aim of the study: The aim of this study was to evaluate potential differences in clinical response to Belimumab among patients with distinct cutaneous subtypes. Specifically, the study assessed disparities related to two subtypes of skin disease: specific and non-specific skin manifestations. Another endpoint of the study was to determine, especially among specific manifestations, whether there was a different response to Belimumab in terms of both the magnitude and timing of response.

A secondary endpoint was to determine if the daily dose of prednisone decreases differently among the various cutaneous subtypes of SLE.

Materials and Methods: The study was conducted retrospectively from 2013 to 2024, following patients from the initiation of Belimumab and involving a nationwide cohort of patients with SLE, all treated in lupus clinics. All adult patients treated with Belimumab (intravenous 10 mg/kg or subcutaneous 200 mg weekly) who had active skin and joint manifestations were included in the study (BeRLiSS-neJS). However, only patients with active skin manifestations were analysed in this study (BeRLiSS-Skin). The Italian lupus clinics which participate to the study were asked to complete a dedicated database for consistent remote data collection. Skin-related information was collected by sorting it into subtypes (ACLE, SCLE, CCLE, cutaneous vasculitis, alopecia/lupus hair, livedo reticularis). Patient response to belimumab was assessed using variation of CLASI-A and CLASI-D scores every 6 months from the start of belimumab treatment. Achievement of CLASI-A remission was defined as CLASI-A=0 and was tested at baseline, 6, 12, 24, 30, 36 months of follow-up. Both parametric and non-parametric tests were appropriately used for analysis.

Results: The study included patients with active cutaneous SLE from 14 Italian centres, with a mean follow-up period of 31.6 ± 20.8 months. Mean age at diagnosis was 29.9±13.2 years. At belimumab initiation 242 patients (54,6%) had skin involvement of which: 112 acute (46,3%), 54 subacute (22,3%) and 18 chronic (7.4%), 48 cutaneous vasculitis (19.8%), 23 livedo reticularis (9.5%), 79 alopecia/lupus hair

(32,6%). CLASI-A score decreased from baseline at 12, 24, and 36 months in all phenotypes. A statistically significant decrease in CLASI-A from baseline was observed as early as 6 months for the acute ($p<0.001$) and subacute phenotype ($p<0.001$), as late as 12 months for the chronic one (0-6 months $p=0.297$, 0-12 months $p=0.003$) and as late as 18 months for livedo reticularis (0-12 months p=0,066, 0-18 months p=0,027). No significant decrease in CLASI-A was found for the other nonspecific skin manifestations of SLE. The variation of CLASI-D showed stability at 36 months compared to baseline for all specific skin phenotypes ($p=0.508$ for acute, $p=1.000$ for subacute, $p=0.770$ for chronic). Among all skin phenotypes, including both specific and non-specific manifestations, no significant decrease emerged in terms of CLASI-D improvement ($p=0.089$). Moreover, remission tested with CLASI-A was more frequent in patients with acute than subacute and chronic phenotype at 18, 24 and 36 months. Finally, this study also demonstrated that the GCs sparing effect of belimumab is significant for patients with acute and subacute lupus, whereas for the chronic subtype, cutaneous vasculitis, livedo reticularis and alopecia/lupus hair subtypes, the variation in daily average PDN intake did not yield significant results. Furthermore, after 36 months of belimumab treatment, considering cutaneous subtypes in the Padua cohort of patients, it was observed an increase of patients with PDN intake < 5 mg/day. Among the specific subtypes, ACLE achieved the greatest reduction in daily PDN intake: at baseline the PDN intake was 0 mg/day only in 8,33% of cases; 0.1-5mg/day in 36,11% of cases, 5.1-7.5mg/day in 5,56% of cases and >7.5mg/day in 50% of cases. After 36 months of belimumab treatment, the intake of PDN was 0 mg/day in 54,55% of cases and 0.1-5mg/day in 45,45% of cases, no patient was taking 5.1-7.5mg/day and >7.5mg/day.

Conclusions: belimumab was effective at reducing cutaneous activity. Significant CLASI-A reduction was achieved later (12 months) in CCLE patients than those with ACLE and SCLE (6 months). CLASI-D stability hints at a lessened damage accrual over the span of 36 months across all skin specific phenotypes. Patients with acute skin subtype achieved easily CLASI-A remission than those with other phenotypes. Finally, belimumab led to a reduction in GCs daily dose in all patients with cutaneous disease, but with significance in the acute and subacute subtypes.

RIASSUNTO

Background: L'efficacia del belimumab nel LES è stata ampiamente dimostrata sia da studi randomizzati controllati, che da studi osservazionali in real-life. Tuttavia, non esistono studi in "real-world" in cui i pazienti siano stati stratificati per sottotipo di manifestazione cutanea.

Scopo dello studio: Lo scopo principale di questo studio era di valutare eventuali differenze nella risposta clinica al Belimumab tra pazienti con diversi sottotipi cutanei. In particolare, sono state esaminate le disparità relative alle due categorie di manifestazioni cutanee: specifiche e non specifiche. Un altro obiettivo dello studio era di determinare, soprattutto tra le manifestazioni specifiche, se vi fosse una risposta diversa al Belimumab in termini di entità e di tempistica della risposta al farmaco. Un'altra domanda, a cui questo studio ha cercato di dare una risposta, è se la dose giornaliera di prednisone diminuisse in modo diverso tra i vari sottotipi di LES cutaneo.

Materiali e metodi: Lo studio è stato condotto retrospettivamente dal 2013 al 2024, seguendo i pazienti dalla prima somministrazione del belimumab e coinvolgendo una coorte nazionale di pazienti con LES, tutti trattati presso cliniche specializzate nel lupus. Sono stati inclusi nello studio BeRLiSS-neJS tutti i pazienti adulti trattati con belimumab (10 mg/kg e.v. o 200 mg s.c.) che presentavano manifestazioni cutanee e articolari attive. Tuttavia, sono stati analizzati solo i pazienti con manifestazioni cutanee attive da cui è nato questo studio (BeRLiSS-Skin). Ai centri italiani che hanno partecipato allo studio è stato chiesto di completare un database dedicato per una raccolta dati sistematica a distanza. I dati sono stati raccolti suddividendo le manifestazioni cutanee in sottotipi (ACLE, SCLE, CCLE, vasculite cutanea, alopecia/capelli lupus, livedo reticularis). Mentre la risposta dei pazienti al belimumab è stata valutata utilizzando la variazione dei punteggi CLASI-A e CLASI-D ogni 6 mesi dall'inizio del trattamento con belimumab. La remissione è stata definita come CLASI-A=0 ed è stata testata a 6, 12, 24, 30, 36 mesi di follow-up. Per l'analisi sono stati utilizzati test parametrici e non parametrici in modo appropriato.

Risultati: Lo studio ha incluso pazienti con LES cutaneo attivo provenienti da 14 centri italiani, con un periodo medio di follow-up di 31,6±20,8 mesi. L'età media alla diagnosi era di 29,9±13,2 anni. All'inizio del trattamento con belimumab, 242 pazienti (54,6%)

presentavano manifestazioni cutanee, dei quali:112 ACLE (46,3%), 54 SCLE (22,3%) e 18 CCLE (7.4%), 48 pazienti con vasculite cutanea (19.8%), 23 pazienti con livedo reticularis (9.5%) e 79 pazienti con alopecia/lupus hair (32,6%). Il CLASI-A è diminuito rispetto al basale a 12, 24 e 36 mesi in tutti i fenotipi. Una diminuzione statisticamente significativa di CLASI-A rispetto al basale è stata osservata già a 6 mesi per i fenotipi acuto ($p<0.001$) e subacuto ($p<0.001$), mentre la diminuzione di CLASI-A per il fenotipo cronico si è vista a 12 mesi (0-6 mesi $p=0.297$, 0-12 mesi $p=0.003$) e addirittura a 18 mesi per livedo reticularis (0-12 mesi $p=0,066$, 0-18 mesi $p=0,027$). Invece, per le altre manifestazioni cutanee non specifiche del LES non è stata riscontrata una diminuzione significativa del CLASI-A. La variazione del CLASI-D ha mostrato una stabilità a 36 mesi rispetto al basale per tutti i fenotipi cutanei specifici ($p=0.508$ per l'acuto, $p=1.000$ per il subacuto, $p=0.770$ per il cronico). Inoltre, tra tutti i fenotipi cutanei (specifici e non-specifici) non sono emerse differenze significative in termini di miglioramento di CLASI-D ($p=0,089$). La remissione è stata più frequente nei pazienti ACLE rispetto a quelli con SCLE e CCLE a 18, 24 e 36 mesi. Questo studio, inoltre, ha dimostrato che l'effetto di risparmio sull'utilizzo dei glucocorticoidi è significativo per i pazienti con lupus acuto e subacuto, mentre per i sottotipi cutanei: cronico, vasculite cutanea, livedo reticularis e alopecia/lupus hair la variazione delle medie della quantità giornaliera di PDN non ha dato risultati significativi. Inoltre, dopo 36 mesi di trattamento con belimumab, nei pazienti della coorte di Padova, è stato osservato un aumento di coloro che assumevano meno di 5 mg/die. Nel sottotipo acuto, al basale l'assunzione di prednisone era 0 mg/die solo nel 8,33% dei casi; 0,1-5mg/die nel 36,11%, 5,1-7,5mg/die nel 5,56% e >7,5mg/die nel 50%. Dopo 36 mesi belimumab, l'assunzione di PDN è stata di 0mg/die nel 54,55% dei casi e 0,1-5 mg/die nel 45,45% dei casi, nessun paziente assumeva 5,1-7,5mg/die e >7,5mg/die.

Conclusioni: Il belimumab è efficace nel ridurre l'attività cutanea. Una significativa riduzione di CLASI-A è stata tardiva (a 12 mesi) nei pazienti con CCLE rispetto a quelli con ACLE e SCLE (6 mesi). La stabilità di CLASI-D suggerisce un ridotto accumulo di danno nel corso dei 36 mesi di trattamento in tutti i fenotipi cutanei. Gli ACLE raggiungono più frequentemente la remissione. Infine, il belimumab ha portato alla riduzione di glucocorticoidi in tutti i pazienti con malattia cutanea, ma in maniera significativa nei sottotipi acuto e subacuto.

SYSTEMIC LUPUS ERYTHEMATOSUS

1. DEFINITION

Systemic lupus erythematosus is a chronic and multisystemic autoimmune disease classified as a systemic connective tissue disorders (CTD). These are characterized by the presence of a systemic, multi-organ inflammatory process, that could potentially affect all organs and tissues. The clinical manifestation of the disease can range from mild to very severe, significantly impacting the prognosis of affected people. Autoimmunity occurs in predisposed individuals, usually after exposure to a triggering agent. The disease leads to the loss of immunological tolerance and activation of the immune system towards self-antigens, resulting in the expansion of autoreactive cell clones (1,2). As a result, the antibodies produced are responsible for tissue and organ damage, which leads to further damage, loss of work productivity, lower quality of life and increased mortality.

2. EPIDEMIOLOGY

A recent study considered the incidence and/or prevalence of SLE in 39 countries worldwide, analysing 112 studies, mostly from high-income countries. Therefore, the incidence and prevalence in low-resource countries remain unclear, both due to methodological and practical difficulties in data collection.(3)

Figure 1: Number of studies distributed by countries.(3)

This epidemiological meta-analysis showed that the global prevalence of SLE is 43.7 cases (ranging from 15.87 to 108.92)/100,000 people, with an affected population of 3.41 millions of people. Regionally, the prevalence of SLE in the general population ranged from 15.9 cases (from 3.29 to 45.85) per 100,000 people in South Asia to 110.85 cases (from 26.74 to 314.1) per 100,000 people in tropical Latin America.(3)

Moreover, prevalence of lupus varies among different ethnicities. African-Americans have the highest incidence rates (4) and it represent a risk factor for the disease, followed by the Asian and Hispanic populations, and finally Caucasians. (5) Furthermore the disease tends to have an earlier onset age and increased severity in African-Americans. (5)

Italian epidemiological data is aligned with global research and shows an incidence of SLE around 40-71 cases per 100,000 inhabitants. (6)

Moreover, SLE predominantly affects women of childbearing age, with a risk of disease incidence decreasing significantly after menopause. The female-to-male ratio

is 9 to 1. Although SLE is considered rare in men, it does tend to manifest more severely. Additionally, men tend to exhibit more frequent skin related symptoms, as well as thrombosis, hypertension, cardiovascular manifestations, vasculitis, cytopenias, renal involvement, neurologic disorders, and serositis compared to women.

Age also plays a significant role in SLE diagnosis. Despite the diagnosis of SLE is being more common in women of childbearing age (20-35y), it has been well-documented in both paediatric and elderly populations. When diagnosed in childhood, SLE is more severe with a high incidence of malar rashes, hematologic abnormalities, hepatosplenomegaly, nephritis, and pericarditis. In older individuals, it tends to have a more gradual onset and is associated with more pulmonary and serositis involvement with fewer occurrences of Raynaud's phenomenon, malar rashes, nephritis, and neuropsychiatric complications.(5)

3. ETIOPATHOGENESIS

3.1. General

The pathogenesis of the disease begins with a genetically predisposed individual encountering a triggering environmental factor (7).

3.1.4. Genetic factors

Genetic background is important in lupus, several observational studies on twins have shown a higher frequency of SLE in homozygotes than in heterozygotes. Furthermore, at the familiar level, it has been observed that 10-16% of patients have relatives with SLE or carrier of other autoimmune diseases.(6)

A combination of genome-wide association studies (GWAS) revealed that both major histocompatibility complex (MHC) and non-MHC genes were found to be linked with SLE susceptibility; however, highly penetrant mutations are not primarily responsible for the pathogenesis of SLE. (2) In fact, the complete complement fraction I1q, C2, C4A, C4B and type IIIB deficiency of FcγR receptors (low-affinity receptor of the Fc fragment of IgG) or mutations in the DNA exonuclease called TREX1 (three-prime repair exonuclease) are responsible for no more than 1-2% of SLE cases. (2) On the other hand, it has been observed that genetic susceptibility to SLE is mainly determined by the interaction of rather common genetic variants, each of which may only slightly increase the risk of the disease. Therefore, the genetic background of SLE patients boasts considerable variability, yet the analysis of the genes identified so far suggests that SLE patients have an immune system predisposed to an aberrant response.

Genetically, it's interesting to note that the risk of SLE is 14 times higher in patients with Klinefelter syndrome (47,XXY), suggesting some association with the X chromosome.(1)

Epigenetic modifications also play an important role in the complex and multifactorial pathogenesis of SLE. Epigenetic mechanisms are sensitive to external stimuli, so environmental factors may play a role in regulating epigenetic modifications.

The main mechanisms of epigenetics are DNA methylation, histone modifications, and microRNA (miR) interference, which modulate chromatin architecture and enable gene transcription or cause gene silencing(2). Abnormalities in both DNA methylation and histone modifications have been reported in SLE.(2)

These epigenetic changes are stable but reversible and are cell-specific but inheritable. This could explain, at least in part, why full concordance is not found in SLE among homozygotic twins, although it is greater than which found among dizygotic twins or siblings (24-57% versus 2-5%).(2,6)

3.1.2. Environmental factors

The most significant environmental factors are:

- Sunlight which can exacerbate SLE. UV rays and UV exposure are triggers for SLE as they lead to increased cellular apoptosis (5).
- Drugs as they can be responsible for causing drug-induced lupus (DILE)(8). Examples of this are procainamide and hydralazine. Sulfa-drugs can also cause flares in patients with SLE (5). It has also been observed that patients taking IFNα for HCV treatment developed symptoms akin to SLE (8).
- Female sex hormones like oestrogen and prolactin, which actually promote autoimmunity and increase B-cell activation. It isn't uncommon to observe during pregnancy the onset of lupus or a disease flare in previous disease in clinical remission (2). Supporting the hormonal influence, data on disease reactivations confirm that women experience more flares than men, precisely because female hormones promote the initiation and maintenance of lupus activity (9).
- Smoking and vitamin D deficiency (1,5,6).

  The association between smoking and SLE seems to have the same mechanism of gene-environment interaction observed in rheumatoid arthritis, including autoimmune trigger factors such as oxidative stress, elevated systemic inflammation, and impaired T- and B-cell function, all smoking-induced.(10) Furthermore this factors interact generally with some autoimmune susceptibility genes of the patients.(10)

Whereas vitamin D deficiency is associated with SLE due to its immunomodulatory properties.(11)

- Pathogens such as viruses play a pivotal role in the pathogenesis. In particular, the Epstein-Barr virus (EBV) has been identified as a possible factor in lupus development, as elevated IFNα levels are needed to control viral infection (2,5). Both adults and paediatric patients with SLE have a higher prevalence of antibodies against EBV compared to the general population. Viral infections encompass both DNA and RNA viruses; these can be internalized into plasmacytoid dendritic cells (pDCs) and meet intracellular Toll-like Receptors (2,12–14). Once stimulated, Toll-like receptors induce the production of Type 1 α Interferon, a cytokine that should protect us from viral infections; however, since interferon is also a major stimulator of the immune system, in a patient with a particular genetic predisposition, it may promote the development of an autoimmune reaction (15).

3.1.3. IFN's role

Connection between IFN and immune cells

From a normal activation of the immune system an exaggerated activation that self-sustains over time, regardless of the nature of the triggering factor (epitope spreading, cross-reactivity, and bystander activation phenomena may occur) was observed in SLE (6,14). In this context, all immune-cells are stimulated by type I interferon, involving not only innate immunity but also acquired immunity cells such as B and T lymphocytes. This facilitates the loss of individual tolerance, the development of autoimmune phenomena and the production of autoantibodies (2,8,15,16).

The autoantibodies target DNA and/or RNA strands, either alone or in association with proteins. This leads to the formation of immune complexes between autoantibodies and autoantigens containing nuclear material. (14,15,17) These immune complexes can, in turn, stimulate plasmacytoid dendritic cells to produce type I IFN, creating a feedback loop for the production of autoantibodies typical of Systemic Lupus Erythematosus. (12,14)

A focus on type 1 IFN role

There are three types of interferons: type 1, 2, and 3. (2,12)

Type 1 and type 3 play a significant role in protection against viruses, while type 2 has a protective role against bacteria. The type involved in the pathogenesis of SLE is type 1, which presents various subtypes (α, β, κ, ε, ω). (12,14)

Potentially, all cells can produce type 1 interferon upon contact with viral DNA and RNA, but plasmacytoid dendritic cells are the main drivers of massive interferon production in lupus. Once produced, type 1 interferon (in its various forms) interacts with its receptor. (16)

It has been observed that all forms of type 1 interferon transmit their signal through the IFN α receptor 1 (IFNAR1) and IFN α receptor 2 (IFNAR2)(14–16). Signal transduction occurs via the JAK-STAT pathway, leading to the maturation of both plasmacytoid and myeloid dendritic cells and contributing to the development of activated B and T cells (14,17). Dendritic cells are important components of the innate immune system, existing in two types:

- Myeloid DCs, antigen-presenting cells with high phagocytic potential, expressing Toll-like receptors.(2,16)
- Plasmacytoid DCs, playing a crucial role in antiviral immunity. While also antigen-presenting cells, their primary role is to produce large amounts of interferon and induce B cell differentiation into antibody-producing plasma cells, contributing to autoimmunity development.(2,16)

	Type 1 IFN	Type 2 IFN	Type 3 IFN
Role in immunity	Pivotal role in antiviral immunity	Pivotal role in antibacterial immunity	Antiviral immunity
Subtypes	• IFNα: 12 subtypes • IFNβ • IFNκ • IFNε • IFNω	IFNγ	IFNδ: 4 subtypes
Triggers for IFN production	DNA, RNA, enveloped viruses, protozoa and some bacteria	IL12, IL18 cytokines	Viruses
Cells able to produce IFN	• Almost all cells can produce IFN type 1 • Plasmacytoid DCs play a key role in Lupus pathogenesis.	• T cells • NK cells	• Almost all cells can produce IFNγ 1-3 • pDCs and DCs • Hepatocytes (IFNγ 4)
Receptors	All type 1 IFNs signal through IFN-α receptor 1 (IFNAR1) and IFN-α receptor 2 (IFNAR2).	• IFNγ R1 • IFNγ R2	IL-28 receptor
Immune outcome	• DC maturation both plasmacytoid and myeloid • T-cell development • B-cell development	• Macrophage stimulation • Increased antigen presentation • Immune-cell recruitment and differentiation • B-cell regulation	Macrophage and DC differentiation into effector cells T-cell growth and activation

Table I: Interferon role in immune response. Inspired by(13–16,18,19)

The IFN1 pathway

Environmental triggers cause tissue damage with necrosis and apoptosis, releasing both nuclear and non-nuclear autoantigens. Dendritic cells phagocytize autoantigens, producing type 1 interferon via the JAK-STAT pathway, which in turn stimulates myeloid cells, efficient antigen-presenting cells also producing BAFF, IL-12, IL-23, and TNF (2,20). Interferon also stimulates neutrophils and NK cells, further influencing the immune response. (20)

T cells, interacting with APCs, externalize TCRs to activate B cells. In SLE, this process is promoted by an autoantigen, leading to B cell autoantibodies production and immune complex formation. This results in immune-complexes deposition in tissues and initiation of tissue damage.(14,20)

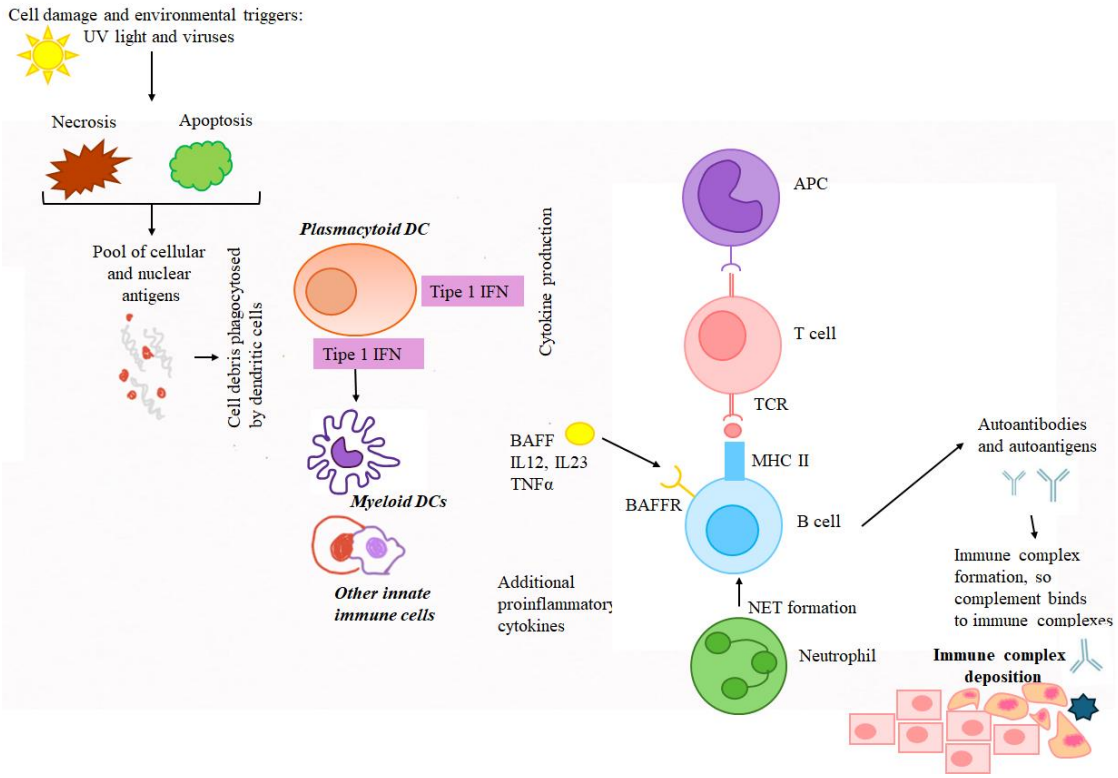


Figure 2: inspired by (14–16,20,21)*, IFN related pathway. IFN effects on pDCs, myeloid DCs, innate immune cells, APC, B and T lymphocyte.*

Role of interferon in cellular damage

Immunocomplex-related tissue damage induces further interferon secretion, fueling this damaging cycle (8). This process underlie both disease onset and its exacerbations.(13,14)

The levels of IFNα correlate with disease activity. In some studies, a relationship between IFNα and disease activity has been observed, suggesting that IFNα may discriminate among patients who generally exhibit higher disease activity compared to other patients with less severe disease(18,19,22,23).

Role of interferon in innate immunity

IFN stimulates NK cells, contributing to tissue damage and induces monocytes to differentiate into myeloid dendritic cells, with ensuing consequences.(14)

Role of interferon in cell-mediated immunity

Activated myeloid dendritic cells communicate with T cells, which act as both mediators of direct cellular damage ($CD8^+$) and of B cell activation ($CD4^+$).(14)

Role of interferon in humoral immunity

In the presence of immune complexes, interferon promotes B cell survival via BAFF production, sustaining B cell clones formation.(24) This type of immunity is often targeted with biological drugs.

Interferon Overexpression in SLE – Interferon Signature

Between 60% and 80% of moderate to severe SLE patients exhibit an interferon gene signature (8,13), indicating overexpression of interferon-regulated genes (25,26). An evaluation of interferon signature levels that categorized patients by disease activity revealed:

- Healthy individuals have a low interferon signature score.(27)
- Patients with mild SLE show intermediate levels of interferon-dependent gene expression.(27)
- Most moderate to severe lupus patients, including those with lupus nephritis, fall into the high interferon signature group.(27)

The data from patients suggest that many of the immunologic and clinical manifestations of SLE might be biologic consequences of IFN-I that is either excessively produced and/ or improperly regulated.(13)

IFN and Clinical Manifestations

- IFN is overexpressed in the skin of lupus patients with cutaneous manifestations (28,29). Notably, keratinocytes also produce type 1 interferon(30), contributing to tissue inflammation and mucocutaneous manifestations. (14)
- Articular overexpression of interferon-induced genes contributes to joint manifestations: synovitis and arthritis.(31–33)
- Plasmacytoid dendritic cells accumulate in the kidneys(7) with inflammatory cell recruitment, interstitial infiltrate, nephron mass loss, hypoxia, and fibrosis. (7,34,35)
- Interferon also increases microglial activity, contributing to local inflammation and psychiatric manifestations. (36)
- Moreover, interferon acts on endothelial cells(7), causing endothelial damage, accelerating foam cell formation and eventually leading to early atherosclerosis. (13,37)
- Exceeding amounts of interferon can lead to cell depletion, therefore reducing infection response capacity.

3.1.4. BAFF/BLyS role in SLE pathogenesis

B lymphocyte stimulator (BLyS) is a ligand belonging to TNF superfamily and it has proven to be a key factor in the selection and survival of B cells. (38) The BLyS protein is a cell surface protein and it's expressed by a wide variety of cell types, including monocytes, activated neutrophils, T cells, and DCs (38). BLyS is than cleaved and released into the circulation. Although standing levels of BLyS are constitutively generated, its expression and secretion can be potentiated by inflammatory cytokines (38). BLyS could bind to 3 receptors:

- BLyS receptor 3 (BR3; also known as BAFF-R), BLyS is the sole ligand for BR3.

- transmembrane activator–1 and calcium modulator and cyclophilin ligand–interactor (TACI)
- B cell maturation antigen (BCMA). (38)

The capacity to bind BLyS emerges concomitant with B cell receptor (BCR) expression, especially with immature B cells appearing from the bone marrow(38). BLyS binding capacity increases through the transitional stages (TR), and the highest expression of BR3 is found in B cells of the follicular (FO) and marginal zone (MZ) (38). The activation of mature B cells depends on TACI and BR3 expression.

Thus B cell development depends on both BLyS stimuli and BR3 expression.

TR stage is the point at which newly formed B cells leave the BM (bone marrow) and enter the circulation and the spleen. At this point, there's the last major checkpoint for elimination of potentially autoreactive primary B cells. BLyS-BR3 interactions promote survival signals, in order to antagonize apoptosis, thus allowing further differentiation into preimmune B cell population. It can be deduced that overexpression of BLyS led to B cell hyperplasia and autoimmune predisposition to develop SLE symptoms.(2,38) This because excess BLyS can cause autoreactive clones, that normally die at this TR checkpoint, to be rescued and allowed to mature. Resuming, these findings imply that BLyS has 2 roles.

- First, it is the key regulator of primary B cell homeostasis, governing the overall numbers of mature, preimmune B cells by controlling their final step of differentiation.
- Second, it plays a central role in maintaining B cell tolerance by balancing anergic cell elimination at the TR checkpoint against the need for additional preimmune B cells.

Normally, this balance is maintained without risks of autoreactive B cell maturation. However, whether B cell production falls outside these norms or whether BLyS levels are unusually high for several periods, autoreactive cells will reasonably increased (2,38). In the last decade, BAFF has therefore emerged as a target molecule, used for the treatment of SLE (2). (see chapter regarding SLE therapy).

3.2. Skin

3.2.1. The role of UV

The skin is composed of a stratified squamous epithelium and keratinocytes are the predominant cell type**.** Keratinocytes migrate from the basal layer to the stratum corneum while differentiating, in a process known as keratinization. From basal to apical epidermidis consist of basal layer, spinous layer, granular layer and cornified layer. Apoptosis is a normal part of keratinocyte development. Under normal conditions, apoptosis occurs in the granular layer of the epidermis. However, external factors can induce premature apoptosis of keratinocytes. Basal cells are more resistant to premature apoptosis induction, while suprabasal keratinocytes are more susceptible to premature apoptosis. (39) It has been shown that UV light can cause keratinocyte apoptosis through multiple mechanisms, including the generation of reactive oxygen species, DNA damage, and the activation of Fas and FasL, among others (39). UV radiation has been shown to induce specific patterns of keratinocyte apoptosis in various subsets of SLE (39).

A high number of apoptotic cells have been demonstrated in the basal zone of the epidermis of DLE lesions (40). In contrast, SCLE lesions showed an increased number of apoptotic cells in the suprabasal layer of the epidermis. (39) Normal skin did not show apoptosis. (39)

In a nutshell, apoptosis along with necrosis are key processes in the pathogenesis of cutaneous lupus lesions.

UV radiation-induced apoptosis also causes the relocation of autoantigens (40). More specifically, UV radiation induces the relocation of SSA/Ro, SSB/La, RNP, and Sm to the cell surface. (39) Antibodies against these antigens are common in SLE.

UV radiation also causes the recruitment of immune cells and the production of cytokine cascades. (39)

UV light is clearly responsible for generating inflammation in both normal skin and skin from patients with LE. The infiltration of the skin by leukocytes and other immune cells in response to UV light is crucial for the development of LE lesions.(39) T lymphocytes are the predominant cell type found in lesions, although pDCs and

myeloid dendritic cells are also increased and, as we have seen before, they play a fundamental role in pathogenesis.

UV radiation mediates the production and release of cytokines and chemokines, promoting inflammation and the recruitment of immune cells (17). More specifically, UVB induces keratinocytes to release IL-1 and TNF-α, primary cytokines in the inflammatory cascade. These cytokines subsequently mediate the release of secondary cytokines. This cascade of cytokines, recruitment of immune cells, inflammation, and tissue destruction ultimately lead to the photoinduced lesions of LE(39).

3.2.2. The role of keratinocytes in the pathogenesis of skin lesions.

Finally, to understand the role of **keratinocytes** in the pathogenesis of systemic lupus erythematosus (SLE) and cutaneous lupus, the following points should be considered:

1. Keratinocytes exhibit an overexpression of pro-apoptotic pathways and a repression of anti-apoptotic transcripts, resulting in an increase of apoptosis processes. E. g. TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) is a pro-apoptotic protein that is increased in the skin of patients with systemic lupus erythematosus (SLE) (41). Additionally, TRAIL-R1, a keratinocyte receptor which mediates TRAIL apoptosis, is also significantly increased (39). Furthermore, TRAIL-R4, a TRAIL receptor with anti-apoptotic properties, appears to be decreased. This suggests that the apoptotic process overrides the anti-apoptotic pathways in the skin of SLE patients.
2. Interferon-α enhances TRAIL expression in keratinocytes, highlighting the interaction between keratinocytes and the plasmacytoid dendritic cells. pDCs are the predominant productors of IFN1α so this can induce a pro-apoptotic environment.
3. Keratinocytes in SLE patients are more sensitive to IL-18, as they express higher levels of IL-18 receptors. Additionally, IL-18 is increased in the epidermis of SLE patients, which appear to be more susceptible to apoptosis when exposed to IL-18. (39)

   IL-12, on the other hand, is a cytokine that protects keratinocytes from apoptosis and it's increased in healthy skin in response to IL-18, while it is reduced in keratinocytes from SLE lesions.(39)

4. Keratinocytes have also been recently found to express type III IFN or IFN-λ(42). IFN-λ shares functional similarities with type I IFNs in terms of antiviral immunity, as seen in previous chapters. Keratinocytes produce high levels of IFN-λ1 in response to immunostimulatory nucleic acids, such as those from apoptotic cells. IFN-λ primarily acts on epithelial cells, and epithelial cells respond by producing pro-inflammatory cytokines like CXCL9, which enhances the recruitment of immune cells. In addition to being enriched in lesional sites, IFN-λ is also elevated in the serum of patients with active lesions of systemic lupus erythematosus (SLE).(22,29,39)
5. HMGB1 is a pro-inflammatory molecule, released by keratinocytes in response to damage, such as UV radiation, or as part of apoptosis in SLE but not in healthy skin(42). HMGB1 increases the production of IFN-α by pDCs and can be absorbed by immune cells and presented in lymph nodes to T and B cells, so this molecule could have a role in enhancing autoimmunity.(39)
6. Keratinocytes are involved in a series of inflammatory stimuli; these are crucial for the development of SLE. Keratinocites are responsible for producing IL-1 and TNF-α, primary cytokines in the inflammatory cascade. This activation leads to antigen-presenting cell activation, induction of adhesion molecules (such as VCAM and ICAM), and recruitment of immune cells.(39,43)

3.3. Autoantibodies

The primary immunological disorder in SLE is the production of autoantibodies. The overproduction of autoantibodies seems to be ascribed to the polyclonal activation of B lymphocytes and the B cell response induced by the antigen (and thus T-cell dependent). The autoantibodies produced are immunoglobulins, directed against certain components of the body, such as those directed against intracellular, nuclear, and cytoplasmic antigens, surface antigens of some cells, or serum antigens.

These autoantibodies produced by B cells can cause damage in various ways, the main ones being:

1. Formation of immune complexes: Autoantibodies are complexed with their respective autoantigens and deposited on tissues, which cause inflammation

through the activation of the complement system and the recruitment of phagocytic cells. The immune complexes to cause damage and thus disease activity must be of a size that allows for entrapment in tissues rather than elimination by phagocytic cells. Additionally, the autoantibodies must be able to resist degradation by DNAse and to have the ability to fix complement. The organ and tissue damage that occurs in the region of IgG deposition is due to infiltration by inflammatory cells, leading to the destruction of tissue organization. Severe damage to multiple organs is one of the leading causes of death in patients with SLE. (37,44). Commonly damaged organs include the kidneys, skin, joints, liver, spleen, lungs, and brain (37,45)

2. Cytolytic action: Some autoantibodies are directed against surface antigens of red blood cells, white blood cells, and platelets and exert a cytolytic type II action (6).
3. Intracellular damage: Additionally, some autoantibodies can enter cells, causing damage through the alteration of vital cell functions (6).

Some studies (46) have shown that the presence of autoantibodies precedes the diagnosis by several years. This discovery could result in facilitating the earlier diagnosis of such diseases; however, what has also become clear is that just detecting these autoantibodies will not be sufficient to predict disease, because the prevalence of, for example, ANA positivity in the general population exceeds the prevalence of SLE or RA.(44,46)

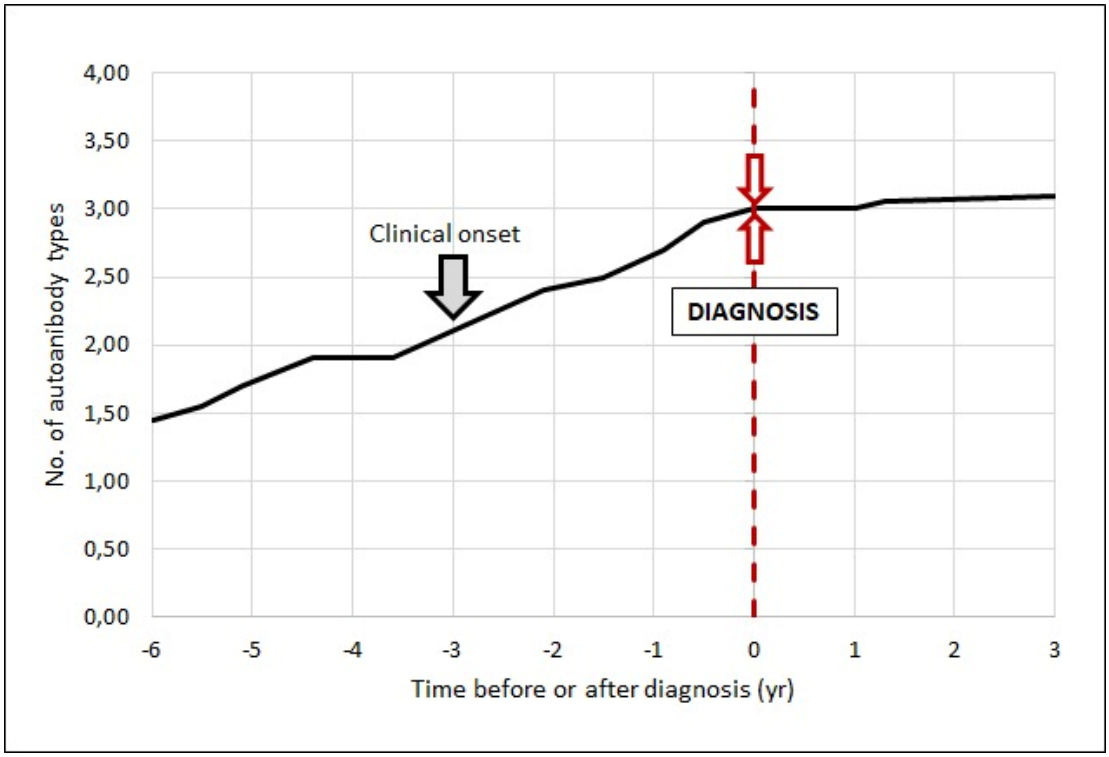


Figure 3: inspired by (46)

3.3.1. Autoantibodies in skin manifestations

Regarding skin manifestations, autoantibodies also play a crucial role: starting from the mechanisms of necrosis and apoptosis (previously discussed), there is an undesirable release of nuclear components, including nucleic acids and other danger-associated molecular patterns (DAMPs), such as the protein HMGB1 (High Mobility Group Box 1). Once released into the extracellular environment, these components become potential autoantigens (47). The accumulated nucleic acids can then be recognized by antigen-presenting cells (APCs) and keratinocytes through pattern recognition receptors (PRRs) (47). Plasmacytoid dendritic cells (pDCs) are the quintessential APCs, and their PRR is the TLR, while in keratinocytes, PRR recognition is mainly considered TLR-independent, although they express them.(37,39,44,47–49)

APCs induce the development and clonal expansion of B and T cells specific to autoantigens. Upon repeated contact with the autoantigen, activated B cells can differentiate into plasma cells to produce autoantibodies specific to nuclear

components, while T cells can migrate to the lesion site to help activate B cells and exert cytotoxic effects against keratinocytes, which in turn leads to the release of endogenous nucleic acids.(47)

Following PRR activation, pDCs and keratinocytes express large amounts of pro-inflammatory mediators (particularly IFN-κ and IFN-λ), as well as other cytokines like various interleukins, tumor necrosis factor (TNF), and BAFF. IFNs then bind to IFN receptors on keratinocytes in an autocrine cycle and induce the expression of IFN-regulated cytokines. The produced chemokines recruit effector cells (CD8+ and CD4+ T cells, pDCs, and macrophages) to the damaged skin. CD8+ T cells can then exert their cytotoxic effect particularly against keratinocytes of the basal epidermal layer, leading to the typical histopathological picture of interface dermatitis.(47)

It is crucial to act as soon as possible on the damage mechanisms, which initially lead to disease activity but can eventually result in induced irreversible damage. (44,45)

However, the predilection for onset at young ages and the potential of the disease to cause irreversible organ damage means that long-term morbidity and premature mortality are high, and many challenges still remain.(44,45)

4. CLINICAL MANIFESTATIONS

SLE is characterized by multiple manifestations which emerge both at the beginning and throughout the course of the disease.(6)

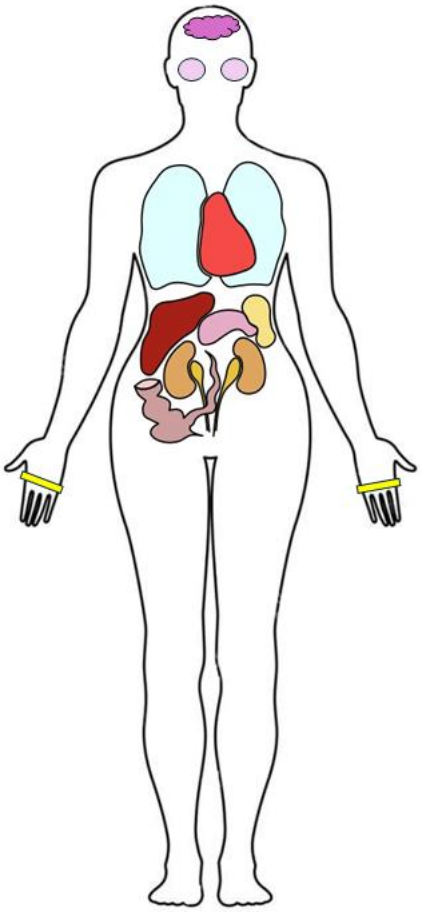


Figure 4: SLE is a multi-organ pathology, the image shows the most frequent organ involvement.

4.1. Constitutional

More than 90% of SLE patients suffer from general or constitutional manifestations, which consist of: fatigue, malaise, fever and weight loss (5,6). Fatigue is often extreme and it's ongoing in more than 80% of patients(6). Fever varies in both magnitude and course and can be caused by the disease itself or by infective complications. Furthermore, approximately 50% of patients experience weight loss.(5,6)

4.2. Mucocutaneous

Skin involvement occurs in almost 60–85% of all lupus patients(45,50,51). Most forms of specific cutaneous lupus manifestations share similar histological findings such as interface dermatitis with perivascular and periadnexal inflammation and may present immunoglobulin and complement deposits at the dermo-epidermal junction (52).
The classification of LE-related skin disease, developed by James N. Gilliam in the 1970s, included specific and non-specific manifestations of lupus (51).

4.2.1. SPECIFIC CUTANEOUS MANIFESTATIONS

Among specific skin lesions there are: acute cutaneous lupus (ACLE), subacute cutaneous lupus (SCLE), and chronic cutaneous lupus (CCLE).(51)

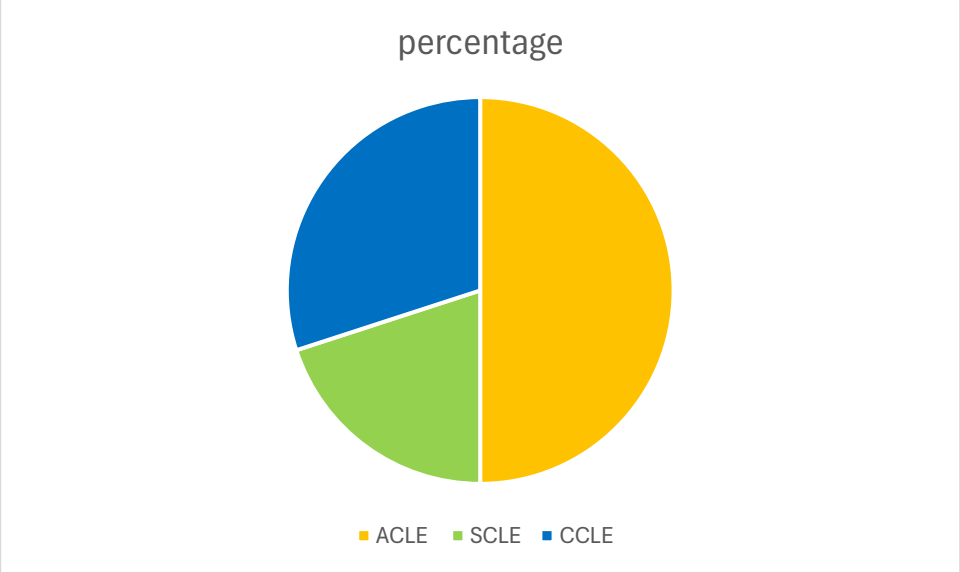


Figure 5: Rates, reported in literature, of acute, subacute, and chronic lupus: ACLE=40-50; SCLE=10-20%; CCLE=15-20%

This different types of LE skin disease share variable relationships with SLE. (51) These relationships are illustrated in figure 3 and will be further discussed below.

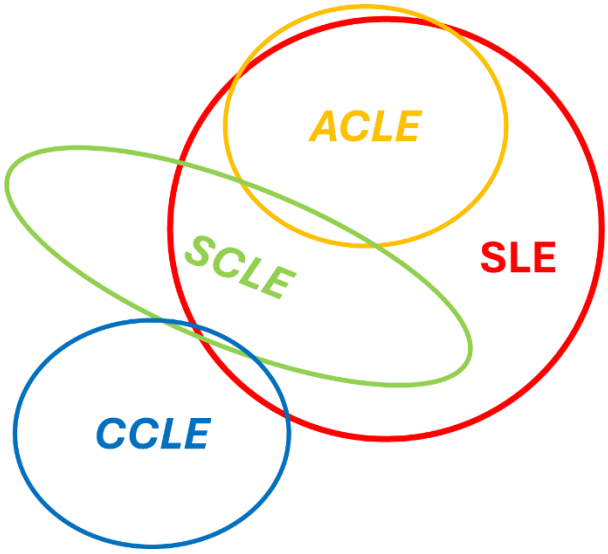


Figure 6: Depiction of relationships between different clinical subtypes of lupus erythematosus (LE)-specific skin disease and systemic LE (SLE). Inspired by (51).

a) Acute Cutaneous Lupus Erythematosus (ACLE)

Acute cutaneous lupus erythematosus (ACLE) is always an expression of systemic lupus erythematosus (SLE), meaning there is always systemic involvement of the disease. ACLE can be localized (most common) or generalized (less common) and it's photosensitive. (5,6)

The typical lesion is the "malar butterfly rash," a localized, erythematous and oedematous rash spreading symmetrically over the nasal bridge and cheeks, sparing the nasolabial folds. (42,53) The butterfly rash generally last from days to weeks, it's often associated with other inflammatory manifestations and it's a sign of active disease therefore it fluctuates with disease activity.(39,42) Moreover, these lesions are usually transient and heal without scarring just when the systemic disease returns under control. (6)

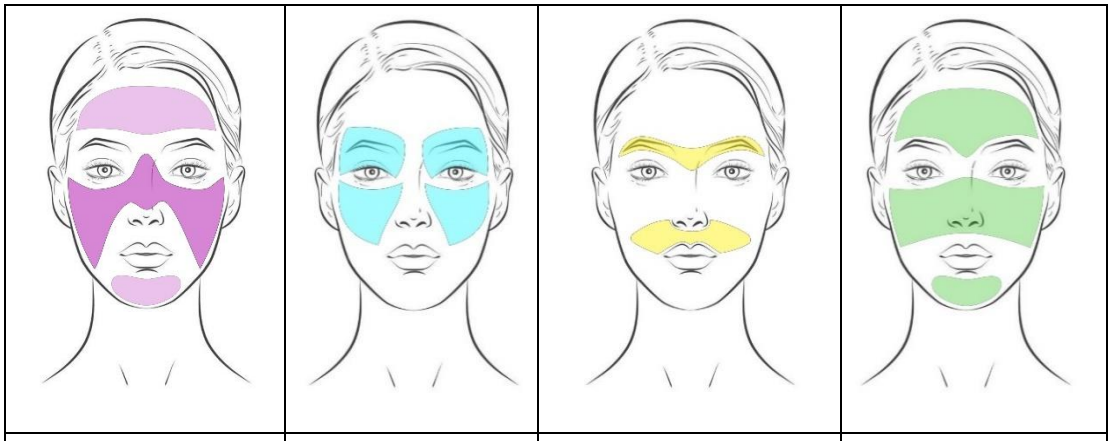
Figure 7: Facial involvement: the intensity of the colour is directly proportional to the frequency of manifestations in corresponding skin area. Hands involvement: if ACLE is generalized, hands are implicated. Inspired by (51,54,55)

The erythema of ACLE in light skin tones manifests as pale pink-to-bright red. Whereas in medium-to-dark skin tones the rash could be more dusky compared to what is observed in light skin tones.(42,55,56) In contrast in dark skin tones ACLE manifests as violaceous erythema with dusky tones and for this reason may go unnoticed to the untrained eye.(55)

This hallmark lesion is often associated with a "palate erythema," indicating mucositis. Likewise cutaneous manifestations are an expression of interface dermatitis, palate erythema underlies interface mucositis.(42,56,57)

Finally, at the conclusion of the active phase, the skin may undergo hyperpigmentation or hypopigmentation. This post-inflammatory dyschromia can persist long after active inflammation has subsided and tends to be more evident in darkly individuals.(51) Indeed, inflammatory rashes in skin of African American descendents are more likely to lead to extensive post-inflammatory pigmentary alteration, just because skin has more melanin in the basal layer of the epidermis. Hence melanin can gathered in the superficial dermis as the process progresses. (51,57,58)

Just to be exhaustive, rosacea, erysipelas, seborrheic dermatitis, and perioral dermatitis must be taken into account in differential diagnosis with ACLE. (6,57)



ACLE	Dermatomyositis	Seborrheic dermatitis	Rosacea

Figure 8: Differential diagnosis. Facial skin has a different pattern of involvement depending on the pathology considered. In particular, SLE (Systemic Lupus Erythematosus) should be included in the differential diagnosis with dermatomyositis, seborrheic dermatitis, and rosacea, which may resemble the rash seen in SLE, but differ in the spatial arrangement of the lesions.(57)

b) Subacute Cutaneous Lupus Erythematosus (SCLE)

Subacute cutaneous lupus erythematosus (SCLE) remains an isolated cutaneous form in 40% of cases, while the residual 60% evolves into a systemic form. It is an extremely photosensitive rash that commonly affects the face, the neck, shoulders, chest, forearms, and extensor parts of the arms. It lasts several months but usually heals without scarring. It is more frequent among White/Caucasian ethnicity, in young women and in smokers.

It is possible to distinguish between two types of skin subacute lesions:

- Papulosquamous (also called psoriasiform skin lesion)
- Annular-polycyclic.

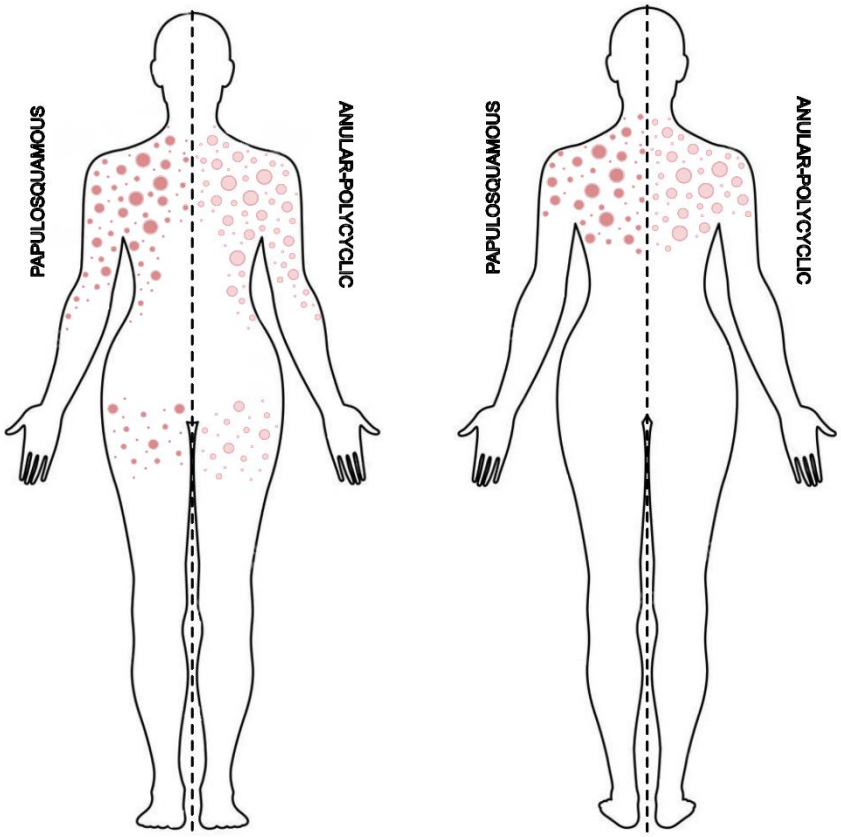


Figure 9: This image represents the two subtypes of SCLE: on the left side, the frontal involvement, on the right side the back view.

Annular SCLE lesions become confluent and produce a polycyclic array, whereas merging papulosquamous SCLE lesions produce a retiform array. Both forms are extremely photosensitive, thus sun protection is tremendously important.

Uncommon clinical variants of SCLE include exanthematous, pityriasiform, exfoliative erythroderma, follicular erythematous and acral annular.(51,58)

SCLE is often associated with anti-SSA antibodies. (59,60)

In differential diagnosis, psoriasis and Tinea Corporis should be considered. (57) The first for its scaly patches, the second because of its annular pattern. Furthermore, Subacute Cutaneous Lupus Erythematosus (SCLE) can be induced by certain types of drugs, hence called drug-induced SCLE.(61) These drugs include PPIs, Hydrochlorothiazide, Terbinafina, Calcium Channel Blockers, ACE inhibitors and NSAIDs. (51,57,58,62)

c) Chronic Cutaneous Lupus Erythematosus (CCLE)

Chronic cutaneous lupus erythematosus (CCLE) typically remains limited to the skin, although in 5-10% of cases it could manifest as a systemic form. (6)

There are several types of CCLE, including:

- Classic Discoid Lupus Erythematosus (DLE) which is the most common form of CCLE and is particularly prevalent among African-American descendents.(57,58) Notably, it can be localized (only head and neck) or generalized (above and below the neck). The lesions start as purplish macules or papules. They are scaly papules very well-demarcated and disk-shaped. These tend to extend into dilated hair follicles, expand centrifugally and deep into the dermis to form a discoid (coin-shaped) plaque. (63)

  In the border area, scales and hyperpigmentation are typically present, as a sign of active inflammation; while in the center of the lesion there's often hypopigmented and atrophic skin, leading to a depressed scar. More than half of the patients will develop significant and destructive scarring, and one-third of patients will develop scarring alopecia.(63) Therefore, after healing, unlike ACLE, DLE leaves depressed scars, atrophy and depigmentation. Moreover, DLE must be differentiated from hypertrophic lichen planus, eczema, actinic keratosis, and psoriasis. (56,57,63)
- Hyperkeratotic/Verrucous DLE characterized by hyperkeratotic (verrucous) lesions. The most commonly affected body regions are: the extensor surface of the arms, face, and hands. It may histologically mimic keratoacanthoma, hypertrophic lichen planus, and squamous cell carcinoma.(61)
- Chilblain lupus erythematosus (CHLE) which is a subtype triggered or exacerbated by cold and humidity, because of that, it appears on the fingers and/or toes, but may also be visible on the ears and face. CHLE emerge as purple or erythematous tender papules, plaques or nodules. There is also a familiar form marked by mutations in the TREX1 (endonuclease repair) gene.(61)
- LE panniculitis which appears as firm, depressed areas. It's characterized by the involvement of the deep dermis and underlying adipose tissue, presenting as firm, depressed nodules. Only 1-3% of patients will show this clinical variant. Lesions may be located on the face, trunk, proximal extremities, and breasts. Differential

diagnosis in lupus mastitis is with breast carcinoma, while for involvement in other areas, panniculitis must be distinguished from T-cell lymphoma, with which it shares similarities.(61) A consequence of panniculitis is dystrophic calcification that typically develops within chronic LE profundus lesions.(61)

- Lupus erythematosus tumidus defined by erythematous plaques in photoexposed areas without superficial involvement. (56,61) LE tumidus presents as deeply erythematous, urticarial plaques with minimal surface changes, no follicular plugging, and histologically appears with rich mucin deposition. LE tumidus is more likely to be located on the face and has a strong female predominance; it is the most photosensitive of all chronic cutaneous forms. Patients presenting with LE tumidus are typically ANA negative and rarely show clinical features of SLE.(61,63)

4.2.2. NON-SPECIFIC CUTANEOUS MANIFESTATIONS

Many skin lesions are associated with lupus erythematosus (LE), particularly systemic lupus erythematosus (SLE), but they aren't specific to the underlying pathological process of the disease.(6) This means that the same lesions can be observed in other pathological contexts outside of LE. Some non-specific skin lesions in LE can reflect the activity of SLE (for example, vasculitis).(51) The most common types of non-specific skin lesions in LE include:

a. Alopecia(51)

   Alopecia represents 40-60% of all non-specific manifestations.

   There are three forms of scalp alterations: telogen effluvium, non-scarring alopecia and scarring alopecia:

   - Telogen Effluvium: This can develop concurrently with a flare-up of LE activity. It involves significant hair loss (up to several hundred hairs per day) with the hairs being in the same phase of the growth cycle. It is frequent in LE patients but is not unique to this condition.
   - Non-Scarring Alopecia: This is also common in other forms of LE and can occur in association with systemic treatments for SLE.(51)

- True lupus alopecia or "lupus hair" is reversible and characterized by the shortening of frontal hair, which is irregular and broken, measuring 5-25 mm in length.(51)
- Scarring Alopecia: Occurs in a third or more of patients and is common in discoid lupus erythematosus (DLE). It results from chronic inflammation leading to atrophy and scarring. Discoid lupus can lead to permanent scarring lupus. Smoking is a risk factor for SLE, increases the risk of discoid lupus, enhances cutaneous activity, and reduces the efficacy of hydroxychloroquine. Alopecia occurs in most SLE patients and can involve eyebrows, eyelashes, beard, and body hair.(51)

b. Photosensitivity

Photosensitivity comprises 45-50% of non-specific skin manifestation and it's a diagnostic criterion for SLE. Photosensitivity is very common in all forms of cutaneous LE. The definition reported in the ACR criteria is: skin rash as a result of unusual reaction to sunlight, by patient history or physician observation. (64) This reaction can be described as the outbreak of specific rashes after exposure to UV. (51)

c. Cutaneous Vasculitis

In the context of LE, vasculitis represents 10-20% of all non-specific skin manifestations and it's typically presents as cutaneous leukocytoclastic vasculitis of small vessels. It can be observed in a generalized or acral distribution. The most common clinical presentation is palpable purpura on the lower extremities.(51) Less commonly, medium-vessel vasculitis in the dermis and subcutis can produce painful nodules similar to those of polyarteritis nodosa. However, medium-vessel vasculitis is more likely to present with mononeuritis multiplex, ulcerated skin lesions, and visceral vasculitis. The presence of cutaneous vasculitis can predict the development of lupus nephritis.

d. Livedo Reticularis

Livedo reticularis accounts for 10% of all non-specific skin presentation. Its presence is associated with cutaneous vasculitis. Additionally, patients with both SLE and antiphospholipid syndrome are particularly prone to showing livedo reticularis. However, it is important to note that livedo reticularis is a cutaneous

finding that can be observed in other medical conditions, some of which SLE patients may experience (e.g., cholesterol embolism).(51)

e. Digital Manifestations (6,51,65)
    1. Periungual Telangiectasia: Occurs in 10-15% of SLE patients but is more frequent and characteristic in dermatomyositis and systemic sclerosis, which are other connective tissue diseases.
    2. Raynaud's Phenomenon: Reported in up to 60% of SLE patients, it is also a common manifestation in other connective tissue diseases. Additionally, subungual hemorrhages (resulting from thrombotic microangiopathy) and sclerodactyly can be observed.(51)
f. Other Cutaneous Lesions

    Other skin lesions associated with LE include bullae, rheumatoid nodules, cutaneous calcinosis, anetoderma, thrombophlebitis, erythromelalgia, erythema multiforme, acanthosis nigricans, lichen planus, and leg ulcers. Cheilitis and facial edema have been reported in less than 5% of patients.(1,51)

4.3. Musculoskeletal manifestations

Between 80 and 90% of patients with SLE suffer from musculoskeletal involvement in their disease history, which can range from mild arthralgia to deforming arthritis.(5)

Lupus arthritis is typically a non-deforming, non-erosive, symmetrical inflammatory polyarthritis (NDNE) affecting predominantly the small joints of the hands, knees, and wrists, although any joint could be involved.(5)

Jaccoud’s arthropathy is the most common. It’s due to joint capsule and ligament laxity, leading to non-erosive deformities of hands, including ulnar deviation and subluxation of the metacarpophalangeal joints, which may mimic rheumatoid arthritis. Usually, these deformities are reducible. Although, rarely, these deviations may become fixed.(5)

There are also cases where lupus arthritis becomes erosive. This is called "Rhupus" or "rhupus syndrome" and it’s often due to an overlap with rheumatoid arthritis. In this case joints appear irreversibly deformed. (66)

Furthermore, avascular necrosis (with or without steroid use) can occur in up to 10% of patients suffering from SLE and it’s usually bilateral and involves the hip joints. (5)

Rarely, inflammatory myopathy may occur and its histopathological features are similar but less striking than polymyositis has been seen in less than 10% of SLE cases.(5)

Regarding tendons and bursae, the most frequent alterations are: tendonitis, tenovaginitis and bursitis. (6)

Lastly, patients with SLE are at high risk of fibromyalgia diagnosis, with reported incidences as high as 20%.(5)

4.4. Lupus nephritis

Lupus nephritis is a well-known complication. In SLE patients prevalence of renal involvement ranges from 50 up to 70%.(6,51) Nephritis can affect glomeruli, tubules, interstitium, and vessels. However, the most characteristic manifestations occur at the glomerular level. (6)

Renal involvement represents one of the major causes of morbidity and mortality in SLE.(6,51)

There are six clinical presentation patterns of glomerulonephritis(6):

1. Asymptomatic hematuria
2. Asymptomatic proteinuria
3. Nephrotic syndrome: defined as proteinuria > 3.5 g/day, hypoalbuminemia, and dysmorphic edema (in the lower limbs, dependent areas, and serous effusions).
4. Nephritic syndrome: characterized by hematuria, proteinuria, leukocyturia, cellular casts, hence active urinary sediment, arterial hypertension, increased creatinine, and renal failure.
5. Chronic renal failure
6. Rapidly progressive glomerulonephritis (GN): a form of renal failure which become progressive within a few weeks.

Complicating matters is the lack of correspondence between histological and clinical presentations, as each histological form, with few exceptions, can present with any of the possible clinical scenarios. (5,60,67) Thus, the only way to determine the histological class is through a renal biopsy, allowing for histological analysis and immunofluorescence.(6,68)

Based on glomerular lesions, six histological classes of lupus glomerulonephritis have been described:

1. Class I: Mesangial GN
2. Class II: Mesangioproliferative GN
3. Class III: Focal proliferative GN (involves <50% of glomeruli)
4. Class IV: Diffuse proliferative GN (involves >50% of glomeruli) further classified as:
    1. IV-S Segmental (involves <50% of glomerular tuft)
    2. IV-S Global (involves >50% of glomerular tuft)
5. Class V: Membranous GN (involves the basement membrane)
6. Class VI: Sclerosing GN (an outcome of inflammation)(68)

Biopsy conclusions guide the treatment of lupus nephritis.(60) Prognosis varies for each class, with excellent prognosis for classes I and II and poor outcomes for classes III and IV. (5)Class V usually carries a favorable prognosis except for complications of nephritic syndrome such as thromboembolism, which is extremely common in this class.(5)

Therefore, biopsy is necessary to:

- Determine the aggressiveness of the treatment.
- Evaluate other histological parameters that inform on:
    - Disease activity: e.g. presence of many active lesions indicates the need for aggressive therapy.(5,6)
    - Quantity of chronic lesions: chronic lesions indicate irreversible damage, making therapy futile and the patient having a poor prognosis.(5,6,60)

Lastly, other renal manifestations may include thrombotic microangiopathy, interstitial nephritis, lupus vasculopathy, vasculitis, and arteriolosclerosis.(5)

4.5. Haematologic

The most frequent haematological signs in SLE (Systemic Lupus Erythematosus) are anemia, leukopenia, and thrombocytopenia.(5,60,69)

Anemia (Hb < 11g/dl) appears in at least 50% of patients and can be either immunological or non-immunological. The most frequent type is non-immunological anemia (due to chronic disease, i.e., normochromic normocytic anemia). (6)Other causes of anemia include renal insufficiency, which leads to a decrease in EPO production and consequently a decrease in red blood cell production. Additionally, therapy with cytotoxic drugs can also cause normochromic normocytic anemia. (5,6)

Autoimmune hemolytic anemia, with a positive Coombs test, is found in only 10% of cases and can be a presenting manifestation of SLE.(70)

Leukopenia (WBC < 4000/mm³) is one of the most characteristic and frequent alterations in SLE, specifically in active disease phases.(6) It's mainly due to anti-leukocyte antibodies, but reduced bone marrow production can also contribute to leukopenia. The most frequent white blood cell alteration is lymphopenia (L < 1000/mm³), as a result of the presence of lymphocytotoxic antibodies.(6) However, leukocytosis can also be found, due either to glucocorticoid intake or to infections.

Finally, thrombocytopenia, defined as platelets <150,000/mm³, is attributed to the presence of anti-platelet antibodies, but can also be on account of APS (antiphospholipid syndrome), which can be part of the spectrum of lupus manifestations.(5,6,70) Generally, it isn't accompanied by haemorrhagic manifestations, although purpura or haemorrhages are still possible.(71)

4.6. Pulmonary

Both the pleura and the lung parenchyma can be affected. (6)
Pleuritis is the most common pulmonary manifestation, affecting up to 30% of patients. (5,6,72)Moreover, pleuritis can present as an initial manifestation of the disease. (6,72) The patient reports pain in the costophrenic area, while on physical examination, signs of pleural effusion with possible friction rubs can be noticed. (6) Among the parenchymal manifestations, we find interstitial pneumonias, which include:

- Acute lupus pneumonitis, which is quite rare (4%) and presents with fever, dyspnoea, cough, haemoptysis, and cyanosis.(6) On physical examination, small bubble crackles can be auscultated, especially at the lung bases. Chest X-

ray often shows multiple and bilateral infiltrates.(72) However, these should be differentiated from infections, which are much more common.(72)

- Diffuse alveolar hemorrhage (DAH), which in SLE patients have an incidence of 0.6 to 5.4%, is a feature associated to a high mortality and has therefore a bad prognostic role. However, the range of mortality reported by different series ranges from 0 to 92%(73) Some studies have shown that disease activity is an important factor associated with DAH, alongside with infections including: *Pseudomonas aeruginosa*, *Serratia marcescens*, *Citrobacter freundii*, and *Aspergillus fumigatus*. (74,75) DAH is associated with thrombocytopenia and APS. Diagnosis is based on clinical signs and symptoms which include dyspnea, pulmonary infiltrates, cough, and hypoxemia. (73) The presence of hemorrhagic bronchoalveolar lavage (BAL) or hemosiderin containing macrophages is a criterion for DAH and an increase of carbon monoxide diffusing capacity (DLCO) is possible.(73)

  The use of high-resolution CT usually shows diffuse ground-glass opacities, patches, or consolidations with rapid progress.(73)
- Organizing pneumonia,
- Chronic interstitial pneumonia, this form of pneumonia is characterized by typical honeycomb infiltrates which are well visible, especially on chest CT. It causes restrictive respiratory failure, reduced pulmonary diffusion capacity, reduced compliance and reduced ventilation-perfusion ratio, especially at the bases where the honeycombing is often enhanced.(6)

Less commonly (about 1%), pulmonary hypertension and Shrinking Lung Syndrome can occur.(6) Shrinking lung syndrome is characterized by restrictive respiratory failure in absence of parenchymal changes. It differs from chronic interstitial pneumonia for this reason.(72) This particular manifestation seems to be attributed to the diaphragm which appears weak and easily fatigable. Therefore the diaphragm remains elevated with poor excursion capacity.(6)

Sometimes, pulmonary hypertension may arise, with or without pulmonary embolism, presenting with dyspnoea, cough, and chest pain.(5,6) On cardiac auscultation, it's noticeable the reinforcement and splitting of the second heart sound and a pulmonary

systolic murmur. (6)The possibility of pulmonary embolism should be considered, especially in patients with concomitant APS.(6,76)

4.7. Neuropsychiatric

In SLE, nervous system manifestations could involve both the central nervous system (CNS) and the peripheral nervous system (PNS).(6,67) Additionally, psychiatric manifestations could appear, although the diagnosis may be difficult.

The prevalence of NPSLE varies widely according to different series and is estimated to be between 12 and 95%(36,77)

On one hand, the most common CNS manifestations are refractory headaches and anxiety(6,77). However excluding this features, which appears commonly present in the general population, the prevalence of NPSLE (neuropsychiatric SLE) remains high, reaching 20%. Focal or generalized seizures can occur, often associated with relapse of disease activity. However seizures don't impact negatively the prognosis. Other CNS manifestations include aseptic meningitis, demyelinating syndrome, which incorporates optic neuritis and myelitis. (36,60,77) Finally, patients could experience movement disorders such as chorea, often accompanied by cognitive dysfunction. SLE patients are also at high risk for ischemic strokes.(6,76)

On the other hand, peripheral nervous system manifestations include cranial and peripheral (sensorimotor, axonal) neuropathies, mononeuritis multiplex, autonomic neuropathies, and syndromes that mimic Guillain-Barré syndrome and myasthenia gravis. (6,36,77)

Finally, psychiatric manifestations are difficult to diagnose and manage and can range from depression and anxiety to frank psychosis.(6,60)

4.8. Ocular

Ocular manifestations include keratitis, keratoconjuctivitis sicca (with or without Sjögren syndrome), scleritis, episcleritis, uveitis, retinal vasculitis, retinopathy, occlusion of the retinal artery or vein. Other less common manifestations are optic

neuritis, neuromyelitis, ischaemic optic neuropathy and chiasmopathy in SLE have also been described. Furthermore eyelid disorders, orbital involvement are possible. (60,78). Keratoconjunctivitis sicca is the most common manifestation while retinal and choroidal involvement are most associated with visual loss.(78) Visual prognosis of retinal involvement depends on pattern of retinopathy, and vaso-occlusion usually leads to poor visual outcome.(78)

4.9. Cardiovascular

Cardiac involvement in SLE is quite common. The pericardium, the myocardium, the endocardium, the valvular apparatus and coronary arteries may be affected.(5)

The most common presentation is pericarditis, which could manifests as chest pain exacerbated by movement and breathing. (71)This pain is typically relieved by leaning forward, it may present with friction rubs and sometimes signs of effusion. Pericarditis rarely leads to cardiac tamponade.(6)
ECG findings may include ST segment elevation, but unlike STEMI, it involves all leads (except aVR and sometimes V1). PR segment depression may be appreciated, this depression will return to baseline over days, along with T wave inversion. Finally, the ST segment will return to baseline until normalization.(1,6)
Echocardiography may reveal effusion or thickening of the pericardial layers.

The myocardium is predominantly affected by myocarditis or functional alterations. Myocarditis is rare and is associated with anti-Ro (SSA) antibodies, while functional alterations mainly consist in arrhythmias whitout myocarditis.(5,6)

Regarding myocardial infarction, it may be particularly common in women with lupus. The incidence of MI is from 5 to 50 times higher than in counterparts without systemic lupus erythematosus, between the ages of 45 and 55.(6) In most cases, myocardial infarction is actually due to accelerated atherosclerosis. Atherosclerosis is often found at a young age in these patients, due to immune-mediated damage on the vascular endothelium (as seen previously in the IFN's chapter)(29). Myocardial infarction could also be a result of coronary thrombosis in patients with positive antiphospholipid antibodies.(5,6,62)

Regarding the endocardium, the atypical verrucous endocarditis of Libman-Sacks is uncommonly reported (5). Libman-Sacks vegetations are abnormal tissues which grows around heart valves, marked by an autoimmune, inflammatory and thrombotic pathogenesis.(6) These vegetations can lead to embolic cerebrovascular disease, peripheral arterial embolism, superimposed infective endocarditis, uncommonly it causes severe valve regurgitation with the need of valve surgery. Therefore, accurate detection of Libman-Sacks endocarditis may lead to early therapy and prevention of the development or progression of its associated complications.(6)

4.10. Gastrointestinal

In order of frequency, iatrogenic lesions of the GI tract are certainly the most common in patients with SLE.(5,60) Among these, gastritis and gastric ulcers are particularly common. Other manifestations of the digestive tract are much less common, including:

- Dysphagia, in about 5% of cases, and reduced peristalsis similarly to what occurs in other connective tissue diseases (scleroderma).(6)
- Intestinal involvement may present with enteritis or colitis, which are isolated and quite rare cases.(6)

Pancreatic manifestations such as pancreatitis have also been reported. This seems to be particularly severe in SLE.(6) However, in patients with SLE an increase in serum amylase is quite frequently found, even in the absence of obvious signs of pancreatitis. Furthermore, it's advisable to make a differential diagnosis with iatrogenic pancreatitis, often caused by GCs (glucocorticoids).(6)

The liver is also affected in SLE. The most common manifestations are elevated transaminases and hepatomegaly. These may indicate lupus hepatitis when found during disease activity phases. Alternatively, they may be attributable to iatrogenic hepatopathy when induced by drugs (e.g., ASA, NSAIDs, immunosuppressive drugs, and antimalarials).(6)

Finally, ascites may occur in patients with lupus. This is a direct consequence of three alternatively possible causes: heart failure, nephrotic syndrome, liver cirrhosis. However, if peritoneal effusion is accompanied by other serositis, due to an inflammatory process of the serous membranes, it may be part of polyserositis.(6)

5. TESTS AND DIAGNOSIS

5.1. How to make SLE diagnosis

The diagnosis of SLE is composed of:

1. recognizing clinical manifestation (single or multiple) compatible with SLE;
2. testing the patients for SLE-related autoantibodies compatible with SLE
3. if the previous are present, before diagnosing SLE, excluding lupus mimickers.

This process to obtain the diagnosis is marked by these three steps well represented in the table below.

Manifestation(s)	Autoantibodies SLE correlated	Differential diagnosis to exclude lupus mimickers
Arthritis Malar rash Fever Photosensitivity Raynaud's Serositis Nephropathy Oral ulcers Neurological Thrombocytopenia Lymphadenopathies Hemolytic anemia Myositis Lung involvement	ANA (lack of ANA positivity is very uncommon in SLE) Anti-dsDNA Anti-nucleosome Anti-histone Anti-SSA Anti-SSB Anti-U1RNP Anti-Sm Anti-P Anti-Ku Anti-PCNA Anti-cardiolipin (aPL) Anti-β2GP1 (aPL)	Drug induced lupus Cutaneous mimickers Other autoimmune diseases Infectious diseases Hematological malignances Multiple sclerosis Still's disease

Table II: This image represents the three steps to SLE diagnosis, it was inspired by (79–81)

5.2. Serologic tests and biomarkers

A biomarker is a measurable indicator of a biological process, whether that process is normal or pathological. (82) In addition, it can be an indicator of a response to exposure

to an intervention or drug. Biomarkers are detected by qualitative and/or quantitative testing and can be measured in multiple matrices: in blood, urine, or tissue.(6) Biomarkers play a crucial role in diagnosing SLE, evaluating SLE complications, observing SLE disease activity, and assessing the therapeutic effect of drugs administered to the SLE patient. (82)

Because SLE can cause damage to various organs, has a complex pathogenesis, and has heterogeneous clinical manifestations, a particular biomarker may reflect only a specific aspect of SLE but may not be useful in reflecting the status of the disease as a whole (67); therefore, an integration of information from both the clinic and the testing laboratory must be made. (6,62)

5.3. Biomarkers and autoantibodies for SLE diagnosis

In the last years, with the increase of autoantibody tests, immunologic abnormalities have been found in essentially all SLE patients. In parallel, the presence of immunologic abnormalities has become evident, especially for therapeutic purposes. In fact, the phase II clinical trial of belimumab showed effects only in serologically active patients (83). Furthermore it's now evident that antibodies are present in patient's serum long before the disease becomes clinically evident (13). Additionally, these antibodies have reasonably a prognostic role.(39)

Normally, in order to investigate for SLE, basic and routinary test are (60):

- Complete blood count, in order to evaluate haematological involvement.
- Direct Coombs test (indicated if patient presents with haemolytic anemia and reticulocytosis)
- Erythrocyte sedimentation rate (ESR)
- C-reactive protein (CRP)

ESR and CRP values can be used to monitor disease activity. They increase proportionally and simultaneously in the subgroup of SLE patients with serositis and/or arthritis.

- Comprehensive metabolic panel to estimate CV risk with blood glucose, glycated hemoglobin, total cholesterol, HDL and triglycerides.

- Urinalysis, which includes: urine analysis, urine culture if necessary, creatinine and eGFR, 24-hour urine protein.
- Complement C3 and C4
- Creatine phosphokinase (indicated in patients presenting with muscle weakness)
- Specific serologic tests: ANA (antinuclear antibody) and, if positive, anti-dsDNA (double-stranded DNA), anti-SSA/SSB, anti-Smith/RNP, antiphospholipid antibodies.

5.3.1. Serum ANAs

Antinuclear antibodies (ANAs) are immunoglobulins of mainly G, M and A class, specific for "self" antigens of cell nuclei.(6,84,85) They are therefore autoantibodies directed toward cytoplasmic or nuclear constituents of all cells. (85)It is important to remember that, despite the name, some of these autoantibodies are directed against the cytoplasm(6). At one time it was unclear how it was possible for antibodies directed toward constituents segregated within cells, which never come into contact with the immune system, to form in the body. This concept was later understood with the discovery of apoptosis, as a result of which the nuclear and cytoplasmic constituents of cells are exposed to the outside world, thus coming into contact with the immune system; as a result of this process, an antibody response can be formed under particular conditions.

The methods of ANA determination are(6,85,86):

a. Indirect immunofluorescence(85): a classic test for the evaluation of ANA. Initially it was performed on a tissue and then performed on a cell culture, such as, for example, HEp-2.(86) A patient's serum is placed on the substrate, and if anti-nuclear antibodies are present, they bind to the nuclei of the substrate cells. Through the use of a fluorescent substance, directed toward the Fc fragment of the immunoglobulin, the positivity of the nuclei of these cells is highlighted under a fluorescence microscope. This is the test with the highest sensitivity and, for this very reason, it is still used;

   Immunofluorescence provides insight into several features of ANAs:

   - Fluoroscopic pictures;

- Titre; the presence of an ANA immunofluorescence titre ≥ 1:80 constitutes a mandatory SLE entry classification criterion.
- Immunoglobulin class;
- Antigenic specificity: knowledge of specific antibodies is extremely important diagnostically.(6)

In addition, fluoroscopic pictures identify some specific ANAs, recognize nuclear "organelles," and diversify the clinical value of ANAs. They are distinguished into major and rare. As shown in the image on the right, four main fluoroscopic pictures are distinguished:

- Peripheral picture: characterized by fluorescence at the periphery of the nucleus;
- Diffuse framework;
- Dotted framework;
- Nucleolar framework.

b. ELISA with extractive and/or recombinant antigens(6): later, this enzyme immunoassay method, which exploits precisely extractive or recombinant antigens on a nitrocellulose substrate, was started to be used. To date, new automated methods are being used, which allow many more antigens to be tested in much less time and at less expense; these tests have achieved a good level of sensitivity and specificity. Although immunofluorescence has higher sensitivity, greater specificity in response is achieved by testing antibodies with more than one method. (82)

Although ANAs aren't exclusive of SLE, they are highly characteristic and can be used as biomarkers for screening, classification, diagnosis, prognosis, and staging. (82) ANA tests have high sensitivity, 90% to 95% in SLE patients, but relatively low specificity, as they can occur in 5-20% of healthy controls and in elderly people. The sensitivity of ANA tests may be related to the early diagnosis of SLE.

However, a negative ANA test doesn't exclude the diagnosis of SLE, because ANA-negative SLE patients do exist. (82,87)

Clinicians should test for ANA, and if the result is positive, they should test for antigen-specific ANA, such as those targeting double-stranded DNA (dsDNA) or

ribonucleoprotein complexes (Ro/SSA, La/SSB, Smith, and RNP), collectively referred to as extractable nuclear antigens.(67,82)

There are several possible nuclear and cytoplasmic autoantigens targeted by ANAs. The main subspecificities of ANA antibodies are anti-dsDNA and anti-ENA (extratable nuclear antigens). (6)

5.3.2. Anti ds-DNA

Anti-DNAs are SLE-specific antibodies that target both double-stranded (ds) and single-stranded (ss) DNA. Anti-dsDNA antibodies are considered nonspecific and may be present as a laboratory error or in the healthy population. (1) Anti-dsDNA antibodies have greater than 95% specificity for SLE, but are found in only about 60%-70% of SLE patients. (5) Therefore, a negative anti-dsDNA does not exclude the diagnosis of SLE. (5) As for differential diagnosis, anti-dsDNA antibodies may also be present in drug-induced lupus, mainly secondary to anti-TNF and IFN-α agents. Rarely low titres of anti-dsDNA antibodies have been reported in rheumatoid arthritis and Sjogren's syndrome. Also, in SLE, anti-dsDNA antibodies have been seen to correlate with disease activity and the development of lupus nephritis.(5,82,87) The level of anti-dsDNA antibodies can fluctuate over time because of their association with SLE disease activation, so they increase if the disease flares up (especially in active nephritis) and decrease when it goes into remission. (88)

5.3.3. Antibodies against extractable nuclear antigens (ENAs).

Antibodies against ENAs associated with SLE include:

- Anti-SM (Smith), which are SLE-specific antibodies.(89)Their presence is characteristic and typical in the SLE patient, but their sensitivity is low, detected in 20-30% of SLE patients.(5) These antibodies are often found together with anti-U1-RNP antibodies, because it has been documented that anti-Sm and anti-RNP precipitate in the same snRNP (small nuclear ribonucleoprotein particles) complex, which can then be divided in two classes. The former, which precipitates by both sera, contains RNP antigen

in association with Sm antigen. The latter precipitates only by anti-Sm sera and therefore doesn't contain the RNP antigen.(90)

Anti-Sm antibodies have shown associations with constitutional symptoms, central nervous system disease, and lupus nephritis (87), but they cannot be used to assess disease progression (91).

- Anti-U1-RNP antibodies, are RNA-protein complexes involved in pre-mRNA processing.(92) They may be present in mixed connectivitis and, unlike anti-SM, are not SLE-specific (5) and do not correlate with disease activity.
- Anti-Ro (SSA) and anti-La (SSB) antibodies commonly found in Sjogren's syndrome. They can be found in SLE and be associated with secondary Sjogren's syndrome, congenital heart block, and neonatal lupus (87). In addition, anti-Ro(SSA) antibody is typical of subacute skin manifestations and of photosensitivity (59).

  Ro-SSA antibodies were detected in 72.1% of patients with SCLE, 47.4% of patients with ACLE, and 22% of patients with DLE.(39) It was also noted that patients with ACLE and positive for anti-Ro/SSA antibodies reported significantly greater photosensitivity compared to antibody-negative patients; (39)

  La/SSB antibodies were detected in 27.5% of patients with ACLE, in 36.2% of patients with SCLE and only in 7.0% of patients with DLE.(39) La/SSB are expressed not only in SLE but also in Sjogren's syndrome.

  Although the role of autoantibodies is still unknown, it's possible to cluster some clinical manifestations with common antibodies in patients with various subsets of LE.(93,94)

  For instance, patients with both anti-Ro/SSA and anti-La/SSB antibodies showed the highest prevalence of discoid rash, photosensitivity, and hematological involvement.(39)

  On the other way, patients with anti-RNP, anti-Sm, and anti-aPL antibodies demonstrated a high prevalence of malar rash (82), which is present in the ACLE subgroup.

While these results don't define a role for these antibodies in the pathogenesis of cutaneous lesions, they demonstrate that antibodies could be used as prognostic factors or better as predictors of disease subsets.(82,87,91)

- Anti-P ribosomal antibodies (anti-P), The prevalence of these antibodies is low (< 5%) (5). Anti-ribosomal P and anti-dsDNA antibodies are often found together in the sera of SLE patients. However, nearly 5-10% of SLE patients may have anti-ribosomal P antibodies as an isolated marker in the absence of anti-dsDNA or anti-Sm antibodies, which supports their important role as an additional tool for diagnosis. (92) Furthermore, the presence of anti-ribosomal P antibodies has been associated with several parameters of SLE disease activity, such as decreased complement levels, photosensitivity, malar rash, or increased disease activity scores, being an important marker of lupus flares. (92) In addition, anti-ribosomal P antibodies have been associated with lupus nephritis and renal activity (92); but they are also considered one of the most unique biomarkers of neuropsychiatric manifestations (NPSLE) (1)

Other autoantibodies associated with the disease are:

- Anti-C1q antibodies. C1q is a key activation protein of the classical complement pathway, as it is the protein that interacts directly with immunoglobulins. Hereditary C1q deficiency, despite being rare, has been described as a risk factor for the development of SLE. (92) The presence of anti-C1q antibodies is not exclusive to SLE as they have been found in many different connective tissue diseases and systemic vasculitis. Their levels correlate with active lupus nephritis, for which they have relatively good sensitivity and specificity; (1)
- Antiphospholipid antibodies (aPL), are risk factors for obstetric complications, thrombosis, and SLE-associated antiphospholipid syndrome (SLE-APS).(76,82,91,92)
- Anti-nucleosome antibodies (ANuA)

  The prevalence of ANuA in SLE ranges from 50% to 100% (82). According to in vitro studies, the nucleosome is an important primary antigen in

SLE.(92,95) Nucleosomes are made from an octamer of histones around which DNA is wrapped. Anti-nucleosome antibodies, which bind only to the native nucleosome particle (i.e., the histone octamer) but not to the individual components (DNA and histone), appear in the early stages of the disease. (92) These anti-nucleosome antibodies precede the formation of anti-dsDNA and anti-histone antibodies. The prevalence of anti-nucleosome antibodies is about 70-90% in SLE patients, higher than the positivity of anti-dsDNA antibodies. These are antibodies that are present in the early stage of the disease and seem to correlate with disease activity, even better than anti-dsDNA antibodies. (92)

Therefore, the presence of anti-nucleosome antibodies is highly sensitive and specific for the diagnosis of SLE, especially when anti-dsDNA antibodies are absent. They indeed assume the role of additional markers of disease activity in the evaluation of SLE disease activity. (87)

These antibodies have also been associated with the severity of lupus nephritis in SLE.

However, the positivity of these autoantibodies is not exclusive to SLE but can also occur in patients with systemic sclerosis and mixed connective tissue diseases, as well as the limitations of laboratory tests used for their detection. Taking all these aspects into account, despite their diagnostic power, to date these antibodies have not yet replaced the anti-dsDNA assay.(92,95)

- Anti-histone antibodies

  The main targets of anti-histone antibodies are the five types of histones (H1, H2A, H2B, H3 and H4), which enable DNA organization.(92)

  The prevalence of anti-histone antibodies in SLE patients is low, about 30%. However, antibodies directed against histones H2A-H2B are present in about 96-100% of patients with drug-induced SLE, so they are characteristic of this subtype of patients. moreover, they are antibodies generally present in patients with lupus nephritis, but they are not antibodies exclusive to SLE.(87,92)

Finally, there is also the B-lymphocyte stimulator (BLyS) or B-activating factor (BAFF) assay. BAFF, as said before, is a member of the TNF family and is a key cytokine for B-cell differentiation, maturation, proliferation and survival. (4)

Serum levels of BLyS are increased in patients with SLE and other autoimmune diseases (1,2).There is a significant correlation between circulating levels of BLyS, disease activity and anti-dsDNA antibodies, so BAFF could become a biomarker of disease activity that can predict flare-ups.(2,4)

5.3.4. C3 (serum Complement 3) and C4 (serum Complement 4)

SLE is an immune complex disease, and immune complexes activate complement, thereby decreasing serum protein levels (89). Generally, if decreased production is ruled out (e.g., due to liver disease), decreased complement levels suggest a deposition of immune complexes, which, however, is not specific to SLE.(89) The serum C3 and C4 assay is an important test for monitoring SLE patients. Indeed, decreased C3 and C4 levels may precede a clinically evident flare and correlate with SLE disease activity, especially in SLE complicated by renal or hematologic flares. (82,87) In the final analysis, keep in mind that, to date, in clinical practice, serum C3/C4 and anti-dsDNA are the only useful predictors of disease activity and flare and thus are recommended for monitoring patients with SLE. (96)

5.3.5. Organ specific biomarkers

Organ specific damage in SLE	Biomarkers
Lupus nephritis	Anti ds-DNA Anti-Sm Anti-C1q Proteinuria 24-h urine protein Chemokines (MPC-1; IL-8 …) Cytokines (e.g. TGFβ, IL-17) Adhesion molecules (VCAM-1; ICAM-1)

Skin lesions	AhR ratio Anti-SSA
NPSLE	Lupus anticoagulant antibodies; Anticardiolipin antibodies; Anti-β2-glycoprotein I antibodies Anti-RibP Anti-U1 ribonucleoprotein antibodies Anti-NMDAR (in CSF)
CVD	Cardiac troponin T IgG anticardiolipin antibodies HDL
Thrombosis	Anti-β2 GPI IgM and IgG Anti-CL IgM and IgG LAC

Table III: Inspired by (2,82)

In conclusion, since no single biomarker could be sufficiently sensitive and specific for SLE, multiple biomarkers combined may be useful to evaluate SLE.

This is also to better meet the need to accurately assess disease progression.

5.4. Diagnosis and biomarkers in CLE

The diagnosis of cutaneous SLE depends on the clinical setting and the rash nature.(61)

5.4.1. Skin histopathology

Skin histopathology is qualitatively similar in each subtype of LE-specific skin disease and can be useful to help diagnose LE but not to determine the clinical subtype.(52)

In cutaneous histopathology, the target of damage is that area between the basal layer and the dermis named *dermo-epidermal junction*.(42,52) In skin biopsies, obtained from lupus lesions, there is evidence of interface dermatitis so that the following is observed:

- a lymphocytic infiltrate (normally CD4 T lymphocytes) in the superficial and/or mid-perivasal and periannexial dermis, attacking the dermoepidermal junction, resulting in damage predominantly in this area. (52)
- A keratinocyte damage, represented by vacuolar degeneration of keratinocytes in the basal layer resulting in thickening of the dermo-epidermal junction and mucin deposition in the dermis. (52)
- Lymphocytosis may also be present at the skin adnexa, for instance around the hair follicles. This explains the permanent scarring alopecia, which is therefore an outcome of hair bulb destruction. This is why early diagnosis is important, particularly in the case of chronic lupus erythematosus; the other forms, both subacute and systemic, generally don't leave scarring outcomes.(52,97)

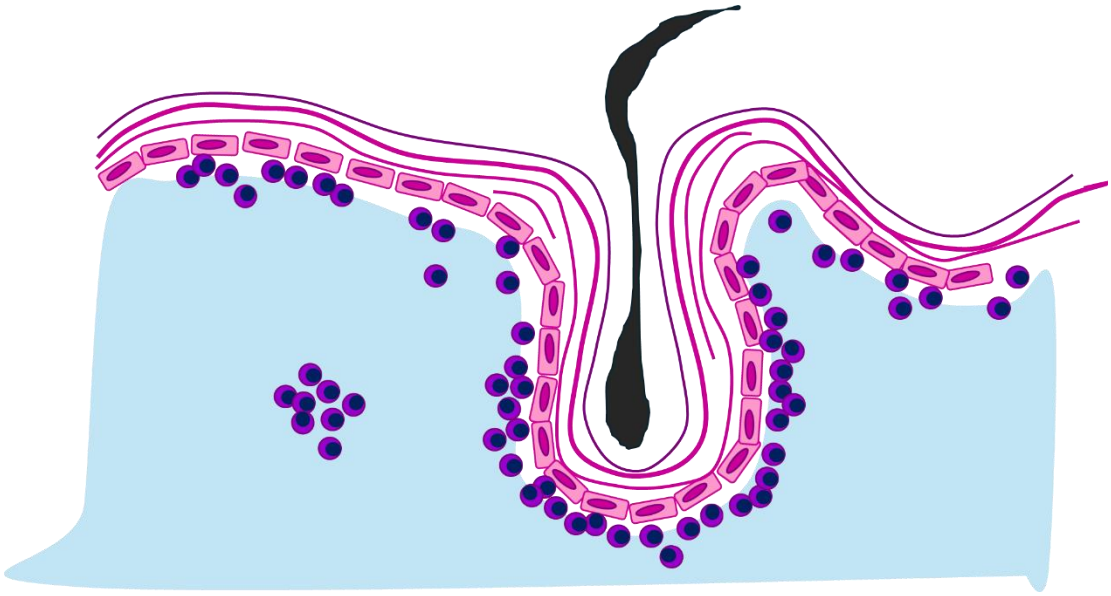


Figure 10: Inflammatory infiltration; image inspired by (52).

What is observed in the histological specimen is a pattern of reaction that should be compared with the patient's clinical situation. Only in rare cases autoimmune diseases have a pathognomonic presentation. Although such skin lesions can be seen to occur as a result of the LE systemic autoimmune process, clinically and histopathologically identical skin lesions are seen in a number of other medical conditions unrelated to LE. (51) To sum up, histology can support the diagnosis, but the concordance with the clinic remains essential.

		ACLE	CCLE
Epidermis	Necrotic liquefactive degeneration of basal keratinocytes.	+++	+
	Hyperkeratosis	+	+++
Dermis	Lymphocytic infiltrate	+	+++
	Edema	+++	+
	Telangiectasia	-	++
Adnexes	Periadnexal lymphocytic infiltrate	+	+++
	Intrafollicular horny taproots.	+	+++

Table IV: Fundamental histological aspects; table inspired by (52).

Furthermore, CLE (Cutaneous Lupus Erythematosus) looks different depending on patient's skin tone. Interface dermatitis appears somewhat dusky in all skin types; however, it may take on a violaceous or purple hue in darker skin tones, compared to more pink or red in light skin tones. The dusky color results from the necrotic keratinocytes in the basal layer of the epidermis. The erythematous to violaceous color is a result of the inflammation.(57)

5.4.2. Immunopathology (51)

In all clinical forms of LE-specific skin disease, immunopathology of injured skin by direct immunofluorescence often shows deposition of immunoglobulins (often IgG) and complement components (often C3) at the dermo-epidermal level. However, these findings may also be present in other connective tissue diseases. Immune deposits are also found at the dermo-epidermal junction in the nonlesional skin of SLE patients.(86) The diagnostic specificity of this finding (nonlesional lupus band test) is highest when three or more immunoreactants are present, and when the specimen is obtained from sun-protected skin.(51) It has been seen that in fact the lupus band test has a low sensitivity (10.5%) but high specificity (97%). (51)

However, the lesional lupus band test is not useful in distinguishing patients with different clinical forms of LE-specific skin disease from those with SLE. For this reason, routine histopathology is generally preferred over direct immunofluorescence to establish the diagnosis of LE. A positive lupus band test is not necessary for the

diagnosis of LE, but it may be useful when other studies are equivocal.(51)In fact, to date how much more simply antinuclear antibodies are preferred to be evaluated to understand whether the patient has a systemic form or a cutaneous form. It is interesting from a pathogenetic point of view to see how at an early stage of disease there are antibodies but not clinical manifestations. Probably the transition between asymptomatic and symptomatic autoimmunity is amplified in individuals destined to develop the disease.(51)

5.4.3. Tests on biological matrices in the patient with CLE:

Evaluation of the autoantibody profile is useful in determining the presence of SLE but has a more limited role in the diagnosis of skin-limited LE.(82) In many cases of SLE, patients initially present with cutaneous findings, so all patients presenting with features of cutaneous LE should be evaluated with a complete history, a systems review focusing on the most frequently involved systems, and a complete physical examination for cutaneous and extracutaneous manifestations(82). In case the skin manifestation is the first to appear, the following should be done:

- Complete blood count,
- Evaluation of liver function
- Assessment of renal function
- Autoantibody profile.(60,82)

Patients presenting with skin manifestations are not always affected by SLE; as seen above, ACLE is most likely to have systemic involvement, whereas in CCLE it is much less likely. In fact, ANA positivity is commonly present in ACLE along with anti-ds-DNA, but in less than 50% of DLEs. Positivity to anti-Sm- and anti-ds-DNA is not commonly found in DLE or SCLE, but is more frequent in ACLE.(61)

Serologic evaluation of autoantibodies therefore helps to distinguish, along with the clinical finding of rash appearance, between an exclusively cutaneous form and a more likely systemic form.

Therefore, serologic testing of ANA and extractable nuclear antigens (ENA) should be performed at baseline to assess possible systemic involvement. (51) Routine blood and biochemical testing, including urinalysis for proteinuria, should be performed. (61) If

antimalarials are considered, a visual inspection should be performed before initiating therapy, (61) since these drugs can give retinal changes.

6. DIFFERENTIAL DIAGNOSIS

Multisystem involvement isn't specific for SLE and can be seen in many other diseases, so called SLE mimickers(98) such as autoimmune diseases, rheumatologic-immunologic conditions, neoplasms, functional disorders, iatrogenic conditions, and infections. (1)

Autoimmune Conditions: in the differential diagnosis, several other autoimmune diseases must be considered. (69)

First and foremost, at initial stages, SLE might appear as undifferentiated or mixed connective tissue disease. In this case, the antibody associated with mixed connective tissue disease is anti-U1RNP.(69)

Rheumatoid arthritis (RA) can be confused with SLE.(99) RA is characterized by intensely inflammatory, erosive (when advanced) polyarticular arthritis, predominantly affecting the wrists and small joints of the hands, and primarily afflicting women. (71) Common features with SLE include symmetrical joint involvement, with joints appearing tender and swollen, along with prolonged morning stiffness. However, the arthritis in SLE tends to be non-erosive (Jaccoud arthropathy and NDNE).(100) Furthermore, RA patients may exhibit extra-articular clinical manifestations, complicating the distinction from SLE, especially in the early stages. Autoantibodies help differentiate between the two diseases: anti-CCP antibodies are typically found in RA patients, while anti-dsDNA antibodies are found in SLE patients.(69,100) There are particular cases where an overlap between the two diseases occurs, where both conditions exist in the same individual. As mentioned earlier, in such overlap cases, the joint involvement in SLE becomes erosive, similar to RA. (66)

Systemic sclerosis, another connective tissue disease, in few cases can resemble the clinical presentation of SLE.(69) This condition is characterized by skin thickening, Raynaud's phenomenon, and microvascular changes visible in nailfold capillaroscopy.(71) Distinguishing between SLE and systemic sclerosis requires antibody specificity testing. Both SLE and scleroderma share anti-nuclear antibodies, but they differ in their specific antibodies. In scleroderma, anti-centromere, Scl-70, and

RNA polymerase antibodies are present, which are not typically characteristic of SLE.(69,71)

Moreover also dermatomyositis must be included in differential diagnosis, due to its autoimmune nature, interferon signature and similar manifestations if compared with those of SLE. So SLE shares with dermatomyositis clinical, immunological, and genetic features but have disease-specific traits. (20) However, in people with systemic lupus erythematosus, almost any organ of the body could be a target for autoimmune inflammation, whereas dermatomyositis, affects predominantly proximal muscles and skin, with erythematous and telangiectatic patches, heliotrope eyelids, erythematous papules of the hand sparing the interphalangeal skin, which indeed appears different to SLE skin manifestations. (81)

Finally, also medium-vessel and especially small-vessel vasculitis should be included in differential diagnosis. Among them urticarial vasculitis and lupus vasculitis must receive special attention.

Urticarial vasculitis (UV) is a condition characterized by chronic or recurrent urticarial lesions that persist for more than 24 hours and may leave residual pigmentation. Patients with UV may have hypocomplementemia, which is associated with more severe disease, systemic lupus erythematosus (SLE)-like manifestations, and a higher prevalence in women.(101)

Histologically, UV shows scattered neutrophilic infiltrates in small vessels, with or without fibrin deposits and extravasated red blood cells. Hypocomplementemic UV also presents with higher positivity in direct immunofluorescence (DIF), which detects deposits of immunoglobulins and complement in the vessels.(101,102)

UV can be associated with connective tissue diseases (CTD) such as SLE and Sjögren's syndrome, complement deficiencies, mixed cryoglobulinemia, and malignancies, but it can also be linked to viral infections, drug reactions, physical exercise, and exposure to sunlight or cold. Therefore, a careful differential diagnosis is required in these cases.(101)

Lupus vasculitis (LV) is a complication that can occur in systemic lupus erythematosus (SLE) or subacute cutaneous lupus erythematosus (SCLE), representing up to 4% of cutaneous vasculitis cases. LV occurs in 19-36% of SLE patients, predominantly affecting the skin (31%) compared to internal organs (6%).(101)

Therefore, LV should be distinguished from other cutaneous vasculitides. Visceral LV, with or without cutaneous vasculitis, should be differentiated from other visceral vasculitides because of its association with higher mortality. Visceral LV potentially exhibits similar features to primary systemic vasculitis.(101)

The most common clinical signs of LV include palmar-digital infarcts that appear as small painful purpuric macules or depressed punctate scars on the palmar surfaces or fingertips, palpable purpura, persistent urticaria, and livedo reticularis of the lower extremities. (101)

Histologically, cutaneous LV often presents as small vessel neutrophilic vasculitis and less frequently as muscular vessel vasculitis similar to polyarteritis nodosa (PAN), from which it needs to be differentiated.(101)

Rheumatologic-Immunologic Conditions: Conditions to exclude in the diagnostic process include viral arthritis, as seen in HIV/AIDS and parvovirus infections. (60) Viral serologic tests, hematologic, and histopathologic tests can aid in distinguishing these conditions from SLE.(60) Sarcoidosis, though an exclusion diagnosis, can resemble SLE, particularly in constitutional symptoms such as cough, dyspnea, fever, fatigue, and night sweats. Sarcoidosis can also present with rash (erythema nodosum) and uveitis.(69) Suggestive findings for sarcoidosis include bilateral lymphadenopathy on chest radiography and non-caseating granulomas on biopsy, along with elevated ACE levels.(71)

Adult-Onset Still Disease is marked by a triad of symptoms: high-spiking fever, arthralgias/arthritis, and an evanescent rash; additional features may include lymphadenopathy, splenomegaly, hepatomegaly, and serositis.(69)

Behçet's Disease: This condition should be considered in the differential diagnosis, particularly for the presence of aphthous genital and oral ulcers, which can be mistaken for SLE-related mucositis.(69) However, uveitis and arthralgias without the systemic and serologic features of SLE strongly suggest Behçet's disease.(69)

Hematologic Conditions: Conditions to consider in the differential diagnosis also include idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, and thrombotic thrombocytopenic purpura.(60) Additionally, autoimmune hepatitis and

autoimmune thyroid diseases can mimic SLE, particularly in their constitutional symptoms.(69)

Antiphospholipid Antibody Syndrome: The antiphospholipid antibody syndrome (APS) can appear in the clinical history of SLE patients, particularly in those with triple positivity for IgG/IgM anticardiolipin (aCL), IgG/IgM anti-β2-glycoprotein I (anti-β2GPI), and lupus anticoagulant (LAC). (51,76) However, APS can also arise independently, without a concurrent SLE diagnosis.(76)

Neoplasms: Hematopoietic cancers and malignant lymphoproliferative syndromes can present with laboratory and clinical findings similar to those of SLE.(91) These conditions are characterized by positive ANA, anemia, low-grade fever, weight loss, pleural effusions, and lymphadenopathy, which can be misdiagnosed as lupus. Special attention is warranted in elderly patients presenting with a new lupus-like syndrome; these patients deserve further investigation and appropriate age-related cancer screening tests.(103)

Kikuchi-Fujimoto disease is also a SLE mimicker; it's a rare condition which consists of a reactive adenopathy due to a massive expansion of CD8+ cytotoxic T lymphocytes. (104)

The cause is not yet known, however, the pathogenesis includes exaggerated immunologic activation of cytotoxic T lymphocytes, that mimics reactive T lymphoma, which is therefore considered in the differential diagnosis of Kikuchi- Fujimoto disease. (104,105)

In Kikuchi-Fujimoto disease the expansion of T cells appears to be aggressive, leading to the destruction of lymph node architecture. The expansion is very rapid, so that the disease manifests as a painful lymphadenopathy, often accompanied by flu-like symptoms. (104,105)

SLE shares with KFD some clinical features such as constitutional symptoms and lymphadenopathy as well as the histological appearance of the lymph nodes. However, serological markers help to distinguish them, for instance, SLE autoantibodies would be notably absent in Kikuchi-Fujimoto disease.(104)

Castleman disease can also mimic SLE; it's a lymphoproliferative process, described as borderline between reactive hyperplasia and lymphoid neoplasia, of which two

clinicopathological varieties has been described: Castleman's hyaline-vascular disease and plasma-cellular Castleman's disease, the latter has similarities with the clinical presentation of SLE, especially because they share constitutional symptoms and multi-organ involvement; in contrast, Castleman's disease has negative results in tests for antibody specificities, which are typically present in SLE (98).

Functional Disorders: Functional disorders like chronic fatigue syndrome and fibromyalgia can present with diffuse musculoskeletal symptoms mimicking lupus. (69) These syndromes can be primary in the absence of underlying autoimmune disease, or they may be secondary to autoimmune conditions, particularly lupus.(60) Fibromyalgia and chronic fatigue syndrome can be distinguished from SLE by the absence of laboratory indicators of SLE and inflammatory musculoskeletal pain.(69) The pain in fibromyalgia is associated with dysperception and oversensitivity to stimuli, as well as muscle stiffness.(71)

Iatrogenic Mimickers: Drugs such as antiarrhythmics (procainamide), antihypertensives (hydralazine), antipsychotics (chlorpromazine), anticonvulsants (carbamazepine), antibiotics (isoniazid, minocycline), anti-inflammatories (sulfasalazine), diuretics (chlorthalidone, hydrochlorothiazide), hypolipidemics (simvastatin), and biological agents (TNFα blockers, interferon α) can cause drug-induced lupus (DILE), a clinical syndrome resembling SLE characterized by fever, serositis, arthritis, and rash.(103) Anti-histone antibodies are detected in about 75% of DIL patients; however, they can also be present in SLE and are not pathognomonic. Anti-dsDNA antibodies are rare in DILE.(103) To properly distinguish between drug-induced and autoimmune etiologies, discontinuation of the suspected drug is recommended. If DIL is the cause, symptoms will subside within days or weeks; if not, the clinical picture will remain unchanged. Less commonly, various drugs can cause drug-induced subacute cutaneous lupus erythematosus (DI-SCLE), characterized by skin lesions in sun-exposed areas associated with anti-SSA antibodies.(52)

Infectious Conditions: Infections, particularly viral ones, are common mimickers of SLE. Common examples include parvovirus B19, CMV, EBV, hepatitis B and C, and

HIV. Rare infections also enter the differential diagnosis, such as bacterial (Treponema p. and Borrelia p.), fungal (Trichophyton), and parasitic (Leishmania and Toxoplasma) infections. Shared features between SLE and infections include fever, cytopenias, rash, inflammatory arthralgias, lymphadenopathy, and ANA positivity(69,103). To differentiate these conditions, it is initially useful to request accurate viral serology, and if the infectious aetiology is still suspected, more specific tests may be warranted.

7. CLASSIFICATION CRITERIA

Classification criteria and diagnosis are two distinct concepts.

On one hand, classification is a methodological and scientific approach that uses a limited number of elements to define and frame a particular disease. (89) The objective is to group relatively homogeneous patients under a single disease definition. (89) Generally, classification criteria are applied in clinical trials and research settings to select a homogeneous group of patients. These criteria require very high specificity and preferably high sensitivity.(62)

On the other hand, diagnosis is an individualized approach devoted to a single patient, generally includes all available information and is often an iterative process based on the exclusion of other entities. (89) Unlike classification, diagnosis is essentially always provisional. For these reasons, classification criteria should not be used to diagnose SLE, as this could result in missing a significant number of patients whose unique disease characteristics do not fit within the homogeneous group defined by the criteria. Instead, classification criteria should be used to conduct scientific studies to achieve standardized results. Diagnostic criteria require both very high specificity and high sensitivity (near 100%), which is very difficult to achieve.(62) This is why there are currently no universally accepted diagnostic criteria.

The American College of Rheumatology (ACR) first developed the SLE classification criteria in 1971 and revised them in 1982 and 1997 (5). The 1982 ACR criteria, revised in 1997, have been widely used for more than three decades.

The ACR has 11 criteria for SLE; if a patient meets at least four of these, SLE can be diagnosed with 95% specificity and 85% sensitivity (69). The criteria include malar or discoid rash, photosensitivity, oral ulcers, arthritis, serositis, abnormal ANA levels, and renal, neurologic, hematologic, or immunologic disorders. (69)

Although the ACR criteria are highly sensitive and specific, patients with mild disease may be missed.(60) Furthermore, ACR classification criteria for SLE do not capture the entire range of manifestations that can be encountered in patients with SLE but focus on the more prevalent manifestations.(62,64) For example, they are highly focused on malar rash but do not include nonspecific manifestations or certain cutaneous manifestations, such as subacute cutaneous lupus and some forms of chronic

cutaneous lupus (lupus panniculitis or profundus and lupus erythematosus tumidus).(62) This presents a disadvantage for the use of these criteria in the diagnostic setting.(62)

Another example is the neurological system, which is very poorly represented in the ACR classification criteria, which includes only two syndromes (seizures and psychosis) and lacks other important syndromes, such as cranial nerve involvement, SLE-associated headache, organic brain syndrome and cerebrovascular accident.

In general, the ACR classification criteria for SLE have more practical value for patients with advanced disease. This can be explained by the fact that the ACR classification criteria require the presence of four or more items to meet the definition of SLE.

In 2012, the SLICC (Systemic Lupus International Collaborating Clinics) revised the ACR criteria to create criteria with increased sensitivity but, unfortunately, not increased specificity. These new criteria added on new items, such as low complement and alopecia classification requires 4 out of the 17 items considered (1), with at least one clinical and one immunologic criterion.(60,106).

The SLICC criteria, which are not limited to research and are widely used as a support for diagnosis, are more sensitive and comprehensive. (67,69,106)

Finally, in 2019 EULAR criteria were developed. (70) These criteria require an ANA of 1:80 or higher as an entry criterion and a total score of 10 is required to classify a patient as having SLE. Indeed, this classification includes 10 domains and 22 criteria, each with weight varying from 2 to 10.(60)

For classification purposes, anti-Ro, anti-La, and anti-U1RNP antibodies were not sufficiently specific for SLE to include them, but they are still useful for diagnostic purposes and routine tests provide reliable results. Similarly, the specificities demonstrated for anti-histone and anti-nucleosome/anti-chromatin antibodies were not sufficiently high for classification, but these antibodies still support a SLE diagnosis, as anti-C1q antibodies do.

	ACR 1982	ACR 1997	SLICC 2012	EULAR/ACR 2019	
				Entry criterion: ANA cut-off title ≥1:80	
Clinical criteria					**Weight**
COSTITUTIONAL				Constitutional Fever	2
MUCOCUTANEOUS	Malar rash	Malar rash	Acute cutaneous lupus OR Subacute cutaneous lupus	Acute cutaneous lupus	6
	Discoid rash	Discoid rash	Chronic cutaneous lupus	Subacute cutaneous OR Discoid lupus	4
	Photosensitivity	Photosensitivity			
	Oral ulcers	Oral ulcers	Oral or nasal ulcers	Oral ulcers	2
			Non-scarring alopecia	Non-scarring alopecia	2
MUSCULOSKELETAL	Arthritis	Nonerosive arthritis	Synovitis	Joint involvement	6
SEROSITIS	Pleuritis	Pleuritis OR Pericarditis	Serositis Pleuritis OR Pericarditis	Serosal Pleural or pericardial effusion	5
	Pericarditis			Acute pericarditis	6
RENAL DISEASE	Persistent proteinuria	Persistent proteinuria	Proteinuria OR Red blood cell cast	Proteinuria >0.5 g/24 h	8
	Cellular casts	Cellular casts		Renal biopsy class II OR V lupus nephritis	10
				Renal biopsy class III OR IV lupus nephritis	10
NEUROLOGIC INVOLVEMENT	Seizures	Seizures	Seizures	Seizure	5
	Psychosis	Psychosis	Psychosis	Psychosis	3
			Mononeuritis multiplex	Delirium	2
			Myelitis		
			Peripheral or cranial neuropathy		

			Acute confusional state		
HEMATOLOGIC	Hemolytic anemia	Hemolytic anemia	Hemolytic anemia	Autoimmune hemolysis	4
	Leukopenia	Leukopenia	Leukopenia OR Lymphopenia	Leukopenia	3
	Lymphopenia	Lymphopenia			
	Thrombocytopenia	Thrombocytopenia	Thrombocytopenia	Thrombocytopenia	4
IMMUNOLOGIC CRITERIA					
IMMUNOLOGIC CRITERIA	Positive LE cell Preparation	Anti-DNA antibodies	Anti-dsDNA antibodies	**SLE-specific antibodies** Anti-dsDNA OR anti-Sm	6
	Anti-DNA antibodies				
	Anti-Sm antibodies	Anti-Sm antibodies	Anti-Sm antibodies		
		Antiphospholipid antibodies	Antiphospholipid antibodies	**Antiphospholipid antibodies** Anti-cardiolipin OR Anti-β2GPI OR Lupus anticoagulant	2
			Low complement (C3, C4, CH50)	**Complement levels**	
				Low C3 OR low C4	3
				Low C3 AND low C4	4
	False positive syphilis serology		Direct Coombs test in absence of hemolytic anemia		
	Antinuclear antibodies	**Antinuclear antibodies**	**Antinuclear antibodies**		

Table V: Classification through ages: ACR 1982, ACR 1997, SLICC 2012 and EULAR/ACR 2019 classification criteria. Common and distinctive features of each classification for SLE. Table inspired by (92)

8. CLINIMETRICS: DISEASE ACTIVITY SCORES

The wide variability of clinical presentations in SLE patients required the development of certain tools.(5) These tools should allow for rapid and homogeneous assessment of disease activity levels and chronicity. Thus, these scores assign different weights to the various clinical manifestations of SLE, according to their severity. It comes so easier to compare patient data from different research centres. Additionally, disease activity scores are deeply useful for defining the prognosis of the disease and evaluating the response to therapy.(6)

Although SLE is a multisystem disease which reveals complexities in creating a unified clinimetric assessment, all the score presented below have been validated and have demonstrated good reliability and efficacy in measuring SLE activity level.

First and foremost, disease activity scores are divided into global and organ-specific. (107)

The former includes SLEDAI, which is an instrument providing a single disease activity score to monitor patients from one visit to the next (108). It is designed to assess disease progressions, remissions and flare-ups. (107)

The latter includes CLASI, BILAG, DAS28. These scores capture the variability of the disease in a specific affected organ.(107)

8.1. SLEDAI-2K

SLEDAI is a global index developed in 1986 and revised by the SELENA group (Safety of Estrogen in Lupus Erythematosus National Assessment Group). It was further updated in 2002 as SLEDAI-2K. SLEDAI evaluates 24 items over the previous 10 days and collects manifestations in 9 organs. (107,109) The difference between SLEDAI-2K and SLEDAI lies in proteinuria, skin rash, stomatitis, and hair loss. Regarding proteinuria, SLEDAI-2K considers a new onset or a recent increase in proteinuria of more than 0,5 g/day. While the original SLEDAI, as well as the SELENA-SLEDAI, considered all levels of proteinuria, not just those > 0.5g/day.(107) Therefore, this variation introduced in the SLEDAI-2K score allows to recognize new, recurrent or persistent proteinuria > 0,5 g/24h.

In addition, in the original SLEDAI, skin rash, stomatitis (mucosal ulcer), and hair loss are supposed to be scored only when new or relapsed, whereas in in SLEDAI-2K and SELENA-SLEDAI are scored even though they are persistently present. (107,109)
These SLEDAI-2K modifications in SLEDAI glossary were introduced to score persistent active disease in the items alopecia, mucous membrane ulcers, rash, and proteinuria(110). SLEDAI-2K is widely used in clinical practice; however, it has the disadvantage of considering only the presence/absence of an item, not bothering its severity(108). Activity categories are based on SLEDAI scores: no activity (SLEDAI= 0), mild activity (SLEDAI 1-5), moderate activity (SLEDAI 6-10), high activity (SLEDAI 11-19), very high activity (SLEDAI ≥20). Furthermore, SLEDAI scores above 5 are associated with a probability > 50% of initiating therapy(107).

Items	Weight
Seizure	8
Psychosis	8
Organic brain syndrome	8
Visual disturbance	8
Cranial nerve disorder	8
Lupus headache	8
Cerebrovascular accidents	8
Vasculitis	8
Arthritis (>2 joints)	4
Myositis	4
Urinary casts	4
Haematuria (>5 RBC/HPF)	4
Proteinuria	4
Pyuria (>5 WBC/HPF)	4
New rash	2
Alopecia	2
Mucosal ulcers	2
Pleurisy	2
Pericarditis	2
Low complement	2

Increased DNA binding	2
Fever (< 38.5°C)	1
Thrombocytopenia (<100.000/uI)	1
Leukopenia (<3000/uI)	1

Table VI: SLEDAI-2K indices with relative weights. Inspired by (109)

8.2. SLE-DAS

SLE-DAS (SLE-Disease Activity Score) is a global activity index created to overcome SLEDAI limits and to improve accuracy.

This tool was designed to provide an appropriate global disease assessment score for clinical settings and to evaluate the outcomes of clinical trials. The items considered in SLE-DAS have been reduced to 17, if compared with 24 items in SLEDAI. Additionally, arthritis, proteinuria, thrombocytopenia, and leukopenia are assessed as continuous variables, whereas the other items are evaluated dichotomously (111).

The SLEDAS has a similar specificity if compared to the SLEDAI's, however its nature, with part of items conceived as continuous, contributes to a higher sensitivity of the SLEDAS in detecting clinical changes over time. SLE-DAS allows to assess some item's severity and this partially overcomes the SLEDAI limitations (111).

Moreover, the SLE-DAS shows a higher performance than SLEDAI in predicting damage accrual and the demonstrated correlation of the SLE-DAS with the PGA and SLEDAI-2K legitimizes its use as a validated activity index.(111)

8.3. PGA

PGA is often considered in clinical practice, although it isn't a true activity index. PGA considers fatigue, which cannot be assessed with the SLEDAI. PGA is also included in the items of many activity scores such as SRI4, BICLA, and LLDAS. (108)

Formally, PGA is a continuous indicator, similar to the visual analogue scale (VAS); therefore, it is an indicator with a 3-point scale described as absent, mild, moderate, and severe (1). The term severe indicates the maximum severity universally considered and unrelated to the patient's experience. Despite being a good evaluation index, it has the drawback of being physician-dependent. In fact, it would be correct for the same

physician to always assess the PGA on a patient. According to the PGA concept, relapse is defined as a modification of at least 1 point in the score within the last 3 months. A mild flare will score 1.0 point, a severe flare up to 2.5 points. Finally a clinical worsening to be significant must reflect an increase of at least 0.3 points in the PGA evaluation (1).

8.4. BILAG

In 1988 the British Isles Lupus Assessment Group (BILAG) introduced an index to measure disease activity in patients with SLE, based on the physician's principle of intention to treat. It was then updated in 2004 to enhance the features of this index. It checks out specific manifestations over the previous four weeks across a total of 9 organ systems: constitutional, mucocutaneous, musculoskeletal, neuropsychiatric, renal, cardiorespiratory, gastrointestinal, ophthalmic and hematological. Activity in each organ system is scored as follows:

- A = most active disease;
- B = intermediate activity;
- C = mild, stable disease;
- D = previous involvement, currently inactive;
- E = no previous activity.

Furthermore, BILAG is used to evaluate flares in SLE patients. A severe flare is defined as a new score of A, and a moderate flare with a score of B.

Whereas a response to treatment is defined by the loss of A and B scores in all systems with no new A or B scores, while a partial response is indicated by the loss of an A score with the persistence or development of one or more B scores.

	03.01.2022	03.07.2022	03.01.2023	03.07.2023	03.01.2024
Constitutional	C	C	A	B	C
Mucocutaneous	D	D	B	B	C
CNS	E	E	E	E	E
Musculoskeletal	C	C	A	B	B
Cardiovascular	C	C	B	C	D
Ocular	D	D	D	D	D
Renal	E	E	A	A	B
Haematological	C	C	A	B	C
Gastrointestinal	E	E	E	E	E

Table VII: in this table at 03.01.2023 is describes in red a severe flare (A) in: constitutional, musculoskeletal, renal and haematolgical domains; is described also a moderate flare (B) in orange in mucocutaneous and cardiovascular manifestations. Moreover at 03.07.2023 partial response to treatment is showed. Inspired by (112)

The BILAG index is more sensitive to change and comprehensive than the SLEDAI, as it can assess both the deterioration and improvement of individual organ systems. This makes it more suitable for evaluating the effects of new drugs in clinical trials, as the extent of disease activity in each organ domain is graded, unlike the dichotomous (present/absent) scoring of SLEDAI. SLEDAI cannot record partial improvement or detect worsening of an existing feature (111,112).
The evaluation of mucocutaneous domain in BILAG differs from the CLASI.

On one hand, in the BILAG mucocutaneous index, both disease activity and damage are considered together (e.g., both inflammatory and scarring alopecia are jointly assessed in the same mucocutaneous score; similarly, malar rash and panniculitis are simultaneously included in a single BILAG descriptor, which is again the mucocutaneous score).

On the other hand, CLASI separates disease activity and damage in order to express and highlight the difference between the two, as disease activity can be treated and regressed, while damage rarely responds to therapy.

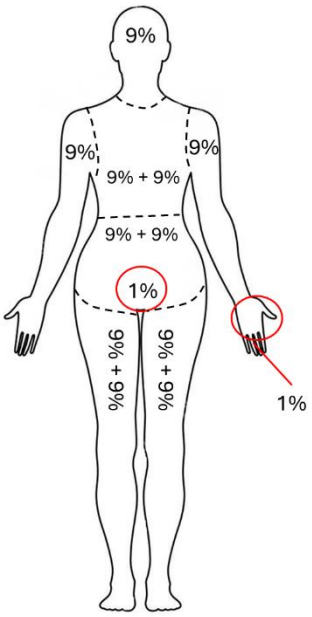
Additionally, unlike CLASI, BILAG proposes a single indicator which represents various areas and extents of disease and includes all shades of severity of manifestations into one single score.

8.4.1. Evaluation of mucocutaneous BILAG index

Category A	Any of the following recorded as 2 (same), 3 (worse) or 4 (new): • Skin eruption - severe • Angio-oedema - severe • Mucosal ulceration - severe • Panniculitis/Bullous lupus–severe • Major cutaneous vasculitis/thrombosis
Category B	Any Category A features recorded as 1 (improving) **OR** Any of the following recorded as 2 (same), 3 (worse) or 4 (new): • Skin eruption - mild • Panniculitis/Bullous lupus - mild • Digital infarcts or nodular vasculitis • Alopecia - severe
Category C	Any Category B features recorded as 1 (improving) **OR** Any of the following recorded as > 0: • Angio-oedema - mild • Mucosal ulceration - mild • Alopecia - mild • Periungual erythema/chilblains • Splinter haemorrhages
Category D	Previous involvement
Category E	No previous involvement

Table VIII: evaluation and summary of mucocutaneous BILAG index. Inspired by (112,113)

8.4.2. Specifications about mucocutaneous items

MUCOCUTANEOUS	
5. Severe eruption  *Figure 11: rule of 9; inspired by (113).*	> 18% body surface area (BSA) any lupus rash except panniculitis, bullous lesion and angio-oedema body surface area (BSA) is estimated using the rules of nines (used to assess extent of burns) as follows: • Palm (excluding fingers) = 1% BSA • each lower limb = 18% BSA • each upper limb = 9% BSA • torso (front) = 18% BSA • torso (back) = 18% BSA • head = 9% BSA • genital (male) = 1% BSA
6. Mild eruption	≤ 18% body surface area any lupus rash except panniculitis, bullous lesion and angio-oedema malar rash must have been observed by a physician and has to be present continuously (persistent) for at least 1 week to be considered significant (to be recorded).
7. Severe angio-oedema	potentially life-threatening eg: stridor angio-oedema is a variant form of urticaria which affects the subcutaneous, submucosal and deep dermal tissues
8. Mild angio-oedema	not life threatening
9. Severe mucosal ulceration	disabling (significantly interfering with oral intake), extensive & deep ulceration must have been observed by a physician
10. Mild mucosal ulceration	localised &/or non-disabling ulceration
11. Severe panniculitis or bullous lupus	Any one: • > 9% body surface area • Facial panniculitis • Panniculitis that is beginning to ulcerate • Panniculitis that threatens integrity of subcutaneous tissue (beginning to cause

	surface depression) on > 9% body surface area panniculitis presents as a palpable and tender subcutaneous induration/nodule note that established surface depression and atrophy alone is likely to be due to damage.
12. Mild panniculitis or bullous lupus	≤ 9% body surface area does not fulfil any criteria for severe panniculitis (for panniculitis)
13. Major cutaneous vasculitis/thrombosis	resulting in extensive gangrene or ulceration or skin infarction
14. Digital infarct or nodular vasculitis	localised single or multiple infarct(s) over digit(s) or tender erythematous nodule(s)
15. Severe alopecia	clinically detectable (diffuse or patchy) hair loss with scalp inflammation (redness over scalp)
16. Mild alopecia	diffuse or patchy hair loss without scalp inflammation (clinically detectable or by history)
17. Peri-ungual erythema or chilblains	chilblains are localised inflammatory lesions (may ulcerate) which are precipitated by exposure to cold
18. Splinter haemorrhages	

Table IX: specifications about mucocutaneous items. Inspired by(113)

8.5. CLASI-A and D

CLASI was developed in 2005 for patients with CLE in order to assess cutaneous manifestations of lupus erythematosus distinguishing disease activity and damage (114). This instrument is regardless of constitutional symptoms and was the first to be approved for clinical trial's usage in CLE evaluation.(115) CLASI was conceived as a single tool to encompass evaluation for the three clinical entities constituting CLE, i.e., discoid lupus erythematosus (DLE), subacute lupus erythematosus (SCLE), and acute lupus erythematosus (ACLE). As said before, CLASI consists of two different complementary scores:

- CLASI-A, which assesses disease activity, summing erythema, scale/hyperkeratosis, mucous membrane involvement, acute hair loss, and nonscarring alopecia.

- CLASI-D, which indicates the damage caused by cutaneous disease on the subject. Damage is scored in terms of dyspigmentation and scarring, including scarring alopecia. Depigmentation is considered irreversible and permanent whether it persists for more than 12 months, doubling the dyspigmentation score.

This separation leads to two scores for each patient.(115) Alternative synthesis of the results into a total score would lead to an unreliable outcome regarding the nature of the skin manifestation, where a summary score of CLASI-A and D- may remain stable while the clinical picture may change completely. (115) In addition, because the current activity or injury may have a major impact on the patient's quality of life and self-esteem, separate scores are preferred (115).

The scores CLASI-A and CLASI-D are calculated by simple addition based on symptoms extension. The CLASI is designed as a table where the rows denote anatomical areas, while the columns score major clinical symptoms. The extent of involvement for each of the skin symptoms is reported according to specific anatomic areas. It’s crucial to know that each anatomic areas are scored according to the worst affected lesion in the area considered. The score is then composed by summing the worst injuries of the various anatomical areas. For a better comprehension, it is recommended to refer to the table below.

	Erythema				**Scale/ Hypertrophy**		
ACTIVITY / Anatomical Location	0	1	2	3	0	1	2
Scalp							
Ears							
Nose (incl. malar area)							
Rest of the face							
V-area neck (frontal)							
Post. neck &/or shoulders							
Chest							
Abdomen							
Back, buttocks							
Arms							
Hands							
Legs							
Feet							

LEGEND	**Erythema**	**Scale / Hypertrophy**
	0 – Absent	0 – Absent
	1 – Pink; faint erythema	1 – Scale
	2 – Red	2 – Verrucous/hypertrophic
	3 – Dark red; purple/ violaceous/ crusted/ haemorrhagic	

+

Mucous membrane
Mucous *membrane lesions (examine if patient confirms involvement)*
0 - absent
1 - lesion or ulceration

+

Scalp	
Recent hair loss (within the last 30 days / as reported by patient)	
0 – No	
1 - Yes	
Alopecia (clinically not obviously scarred)	
0 - absent	
1 - diffuse; non-infiammatory	
2 - focal or patchy in one quadrant	
3 - focal or patchy in more than one quadrant	
Divide the scalp into four quadrants. The dividing line between right and left is the midline. The dividing line between frontal and occipital is the line connecting the highest points of the ear lobe. A quadrant is considered affected if there is a lesion within the quadrant.	
TOTAL ACTIVITY SCORE= add skin, mucous membrane and scalp	

Table X: CLASI-A: regarding disease activity. Inspired by (115)

		Dyspigmentation		Scarring/ atrophy/ panniculitis		
DAMAGE / Anatomical Location		0	1	0	1	2
Scalp						
Ears						
Nose (incl. malar area)						
Rest of the face						
V-area neck (frontal)						
Post. neck &/or shoulders						
Chest						
Abdomen						
Back, buttocks						
Arms						
Hands						
Legs						
Feet						
LEGEND	**Dyspigmentation**	**Scarring/ atrophy/ Panniculitis**				
	0 – Absent	0 - Absent				
	1 – Dyspigmentation	1 – Scarring				
		2 – severely atrophic scarring or panniculitis				

+

Dyspigmentation	
Report duration of dyspigmentation after active lesions have resolved (verbal report by patient)	
0 - dyspigmentation usually lasts less than 12 months	
1 - dyspigmantation usually lasts at least 12 months	

+

Scalp	
Scarring of the scalp (judged clinically). *Divide the scalp into four quadrants. The dividing line between right and left is the midline. The dividing line between frontal and occipital is the line connecting the highest points of the ear lobe. A quadrant is considered affected if there is a lesion within the quadrant.* N.B. If scarring and non-scarring aspects seem to coexist in one lesion, score both.	
0 - absent	
3 - in one quadrant	
4 - two quadrants	
5 - three quadrants	
6 - affects the whole skull	
TOTAL DAMAGE SCORE= add skin, dyspigmentation and scarring of scalp	

Table XI: CLASI-D: regarding disease damage. Inspired by (115)

A positive aspect regarding CLASI is that it hasn't been developed within the framework of a particular clinical trial, because it has been suggested that instruments developed for a particular trial might bias the trial in favor of the treatment. Furthermore, it has been validated for content validity, inter-rater validity, intra-rater validity and practical applicability.
Strengths of CLASI is having been designed as one single instrument for at least the three specific clinical entities that constitute CLE, because it was conceived as an instrument suitable and appliable for all subtypes of cutaneous lupus.

8.6. Revised CLASI (RCLASI)

According to the experts who developed the RCLASI, although the CLASI is a good indicator, it doesn't provide a great description in all subtypes of CLE.(114)
For example, edematous lesions are not included in CLASI evaluation, however, is one of the most prominent features of lupus erythematosus tumidus (LET) (114) and is for this reason it was instead included in the RCLASI.

Furthermore, RCLASI added some specifications regarding lesions in lip area, distinguishing between superficial scaling of SCLE and firm adherent scaling of DLE (114). The dyspigmentation score of the RCLASI distinguishes the hypopigmentation of SCLE from the hypo- and hyperpigmentation of DLE, assigning 1 point each for hypopigmentation or hyperpigmentation, while the simultaneous presence of both assignes a score of 2. Additionally, the RCLASI considers only scarring and atrophy, with a gradient ranging from initial scarring (1 point) to severe, atrophic, vermiculate scarring (2 points). Panniculitis, being more a histological criterion than a clinical one, is excluded in favor of introducing "lipoatrophy" to allow for the assessment of the typical damage in an advanced stage of LE panniculitis (2 points).

The evaluations regarding the mucous membranes have also been modified, as well as the assessment of alopecia with the addition of a new paragraph called "lupus hair" or "vellus hair," a typical manifestation in patients with active disease, characterized by thin, weak, fragile hair, especially along the frontal hairline. Finally, the scarring of the scalp was previously measured in the CLASI by dividing the scalp into four quadrants and assigning points to the number of affected quadrants, even if there was only one

lesion within the quadrant. This method of weighting scalp scarring leads to a high damage score, especially in case of few small lesions scattered across the scalp. RCLASI modified this measurement based on an assessment guideline developed for alopecia areata by Olsen et al(114–116). The percentage of cicatricial alopecia is estimated in each area of the scalp and multiplied by the percentage of the scalp surface involved, resulting in a scale from 0 (absent) to 6 points (75-100% cicatricial alopecia).(114)

8.7. DAS28

DAS28 is primarily used in clinical practice for patients with rheumatoid arthritis (RA). It is an index based on a combination of information, such as laboratory data (ESR or CRP) and clinical criteria (the number and type of joint involvement), accompanied by an overall impression of the patient's well-being or discomfort.(117,118) This index was designed to compare the effectiveness of different treatments on joint manifestations and to evaluate the progression of the disease. Nowadays, DAS28 is used to assess joint involvement in patients affected by SLE.(117)

Specifically, the level of disease activity is defined as low (DAS28 $\leq$ 3.2), moderate (3.2 < DAS28 $\leq$ 5.1), and high (DAS28 > 5.1); finally, remission is defined as DAS28 values lower than 2.6.(117,119)

In a study conducted on SLE patients, DAS28 was demonstrated to be better than SLEDAI-2K in assessing joint disease activity. Up to 50% of SLE patients without joint involvement, as defined by the SLEDAI-2K item, showed moderate to high disease activity according to DAS28 values. (117) These results suggest a greater ability in assessing SLE-related articular manifestations compared to the global activity index SLEDAI-2K.(117,118)

8.8. SRI4

SRI isn’t an indicator of disease activity, whereas is currently one of the most frequently used primary endpoints in clinical trials.(108) This is a composite index which combines SELENA-SLEDAI, BILAG and the physician's global assessment. (108) A responder is defined as: improvement of 4 points or greater in SELENA SLEDAI score

from baseline, matched with no worsening of the doctor's global estimate and without new BILAG A scores or two BILAG B scores.(107) This index is mainly determined by SELENA-SLEDAI and it was used as the primary endpoint in the phase 2 and 3 studies with belimumab (BLISS-52 and BLISS-76) . (120–122)

8.9. SLICC-DI

Irreversible organ damage is a primary outcome in SLE which is caused by both the disease itself and the therapies received by patients.(107,123). The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index (SDI) was developed in 1996 to assess irreversible damage.(123) When the disease is remitting, there are generally no increases in SLICC; however, when the disease flares up, SLICC/ACR-DI score can increase. (107,123) By the way, it’s a score which tends to increase over the course of the disease history of SLE patients. Additionally, damage can reflect the plethora of side effects from the chronic therapies administered to SLE patients. (123)

Damage is defined for 12 organ systems: musculoskeletal (0–7), skin (0–3), renal (0–3), ocular (0–2), neuropsychiatric (0–6), pulmonary (0–5), cardiovascular (0–6), peripheral vascular (0–5), gastrointestinal (0–6), endocrine (diabetes) (0–1), gonadal (0–1), and malignancies (0–2). Damage over time can only remain stable or increase, theoretically up to a maximum of 47 points.(123)

Finally, the SDI also predicts the future mortality of SLE patients.(123) The earlier a patient accumulates damage in their disease history, the lower is their life expectancy.(123)

9. PROGNOSIS

The prognosis of SLE has significantly changed over the past few decades.(6) Currently, the 10-year survival rate is over 90%, mainly due to the introduction of new drugs and the pursuit of new treatments (1,6,44). However, mortality remains 2-5 times higher compared to the general population. (124) Mortality is determined by long-term complications of the disease and by side effects of the treatment. (125) The eminent causes of death in SLE patients are no longer active lupus, but instead cardiovascular disease, complications of renal failure, and malignancy. (125)

The prognosis of a patient is determined by:

1. The clinical phenotype, which reflects the aggressiveness of the disease. We can identify two main phenotypes: mild lupus and severe lupus. In a study on long-term prognosis, it was observed that about one-third of deaths (35.3%) were caused by active manifestations of the disease, including cases with low response to therapy or poor compliance. It is known that corticosteroids and immunosuppressants can induce disease remission in the majority of cases. However, there is a small percentage of patients who do not respond to treatment. These patients, having a severe and not responding to treatment phenotype, are at a higher risk of mortality. Renal involvement is by far the most frequent serious manifestation of SLE. It has been shown to be a serious prognostic factor and thus capable of affecting patient survival.

   The table below illustrates the two major clinical phenotypes in SLE and the related long-term survival plot.

Major clinical phonotypes in SLE	
Mild lupus	**Severe lupus**
Arthritis	Glomerulonephritis
ACLE	Severe NP involvement
SCLE	Acute interstitial pneumonia
CCLE	Chronic interstitial pneumonia
Serositis	Pulmonary hypertension
Leukopenia (1000< WBC<4000)	Myocarditis
	Visceral vasculitis

Thrombocytopenia (15000<PLTs<150000)	WBC<1000/mm^3 PLT<15000/ mm^3 Haemolytic anemia Aplastic anemia

Table XII: Major clinical phonotypes in SLE. WBC= white cell blood count; PLTs= platelets.

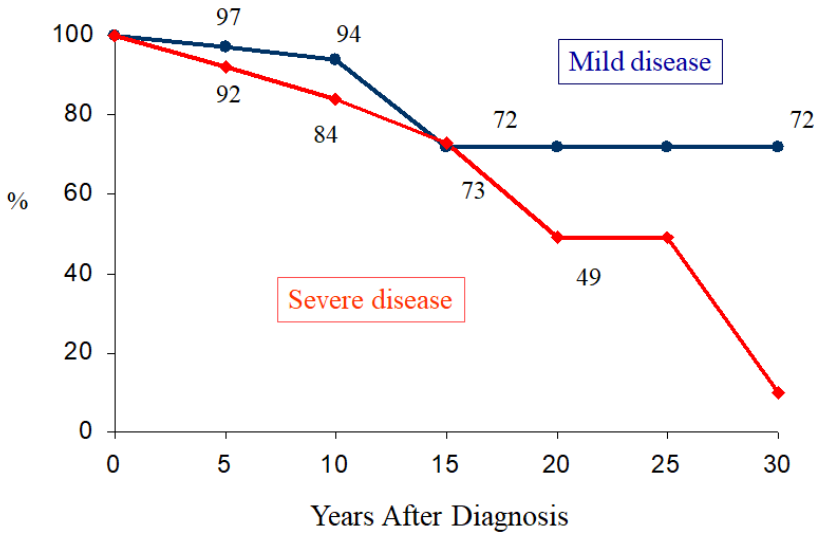


Figure 12: plot of long-term prognosis divided by mild and severe disease. Inspired by (126)

Analysing the survival curves of patients with severe and mild disease, it is noted that the survival curve of patients with mild disease is substantially similar to that of patients with severe disease, but only up to 10-15 years after diagnosis. Subsequently, the curves tend to diverge, showing a clear decline in survival for patients with severe disease.

A possible explanation for this variation in the curves is that the drugs used in the treatment of SLE are effective in controlling even the most severe manifestations of the disease, including lupus nephritis, with few exceptions due to non-responsive or non-adherent patients. Their effectiveness is evident at least up to the first 10-15 years after diagnosis, making the survival of patients with severe disease substantially similar to that of patients with mild disease. However, 10-15 years after diagnosis, new factors emerge that modify the previous profile. These variables could be the result of complex

interactions between numerous environmental factors and disease-related factors. In this sense, severe disease seems to be an important determinant of poor long-term survival because patients with severe disease are more frequently affected by disease complications and exposed to aggressive treatments.

2. Damage accumulation: if disease activity is not adequately treated or is unresponsive to treatments, it leads to damage accumulation, which indeed leads to higher mortality.
3. Pharmacological side effects: the improvement in disease activity and therefore in survival of patients with SLE, particularly in those with severe disease, exposes them to the onset of consequences that can be partly related to the disease itself and partly to its treatment. The use of certain drugs, such as immunosuppressants, corticosteroids, and NSAIDs, has been associated with severe side effects, such as:
    - Infections: usually identified in patients with long-standing disease who had been treated with corticosteroids and immunosuppressants for a long time. These patients are immunocompromised patients with a high risk of infections as a side effect of the therapy.
    - Gastrointestinal bleeding: occurring in patients who had taken GCs and NSAIDs for a long time and were at risk of gastrointestinal bleeding despite proper gastric protection.
    - All those related to continuous intake and medium-high doses of GCs: osteoporosis, hypertension, obesity, metabolic dysfunction, increased glycated haemoglobin, cardiac hypertrophy, amenorrhea in women. The related signs are: muscle wasting in the extremities, enlarged supraclavicular fat pads, moon face, dark facial hair, poor wound healing, abdominal striae.
4. Additionally, the prognosis is also determined by comorbidities associated with lupus, mainly driven by a combination of disease activity, disease duration, and adverse effects from treatments.(127) Comorbidities are mainly due to infections, bone health disfunction, diabetes risk, cardiovascular risk and renal failure.(127) Not only these comorbidities affect a patient's quality of life, but they also add to the challenges in managing SLE, specific screening and

interventions. Finally, the accrual of comorbidities has a direct effect on mortality. (127)

To conclude, two-thirds of deaths are due to disease or treatment complications, while one-third are due to active disease.(127) The latter can be described by flares, remission and patterns.

9.1. Flares definition

SLE patients can experience disease flare-ups and remissions throughout their disease history, often with a fluctuating course. (128) Flare was defined as an increase of SLEDAI-2K≥4 from the previous visit. (128,129) Despite treatment there are still patients who experience flare-ups during treatment.

9.2. Remission definition

Remission in SLE is not clearly defined by any of the available disease activity scores (124). According to SLEDAI, remission may be defined as a SLEDAI = 0, whereas SLEDAI ≥ 4 was referred to as persistent disease activity(124,130).

Remission has three levels which were defined using the SLE Disease Activity Index-2000 (SLEDAI-2K) (131):

- Complete remission: no disease activity in corticosteroid-free and immunosuppressant-free patients.(124,131)
- Clinical remission off corticosteroids: serologically active, but clinically quiescent disease (SACQ) disease in corticosteroid-free patients. (124,131)
- Clinical remission on corticosteroids: SACQ disease in patients taking prednisone 0,1–5 mg/day. (124,131)

Remission is defined as prolonged when lasting ≥5 consecutive years. (131)

Although remission is still difficult to define, an international task force recommended the usage of DORIS definition of remission which consist in: clinical SLEDAI=0 along with Physician Global Assessment <0.5 (0–3). (88)

This definition of remission is regardless of serology and endorsed in patients treated with antimalarials, low-dose glucocorticoids (prednisolone ≤5 mg/day), and/or stable immunosuppressives including biologics.(88)

However, flare and remission alone are insufficient to describe disease activity in clinical trials, because many patients will have long periods of continuous (chronic) disease activity/prolonged remission. For this reason, experts designed models to describe the course of the disease in a specific patient. However, these models have retrospective value, while the future course of the disease remains unpredictable (128,129).

9.3. Definitions of Disease Activity Patterns

In several studies, models have been proposed to reflect the course of the disease by following patients annually and assessing variations with the SLEDAI-2K index.(128)

- Clinically quiescent disease (CQD): a SLEDAI-2K=0 at all three annual visits; this pattern is characterized by the absence of disease activity for at least one year.(129)
- Minimal disease activity (MDA): a SLEDAI-2K=1 at one or more annual visits; this pattern is characterized by periods of minimal disease activity that vary in length. (129)
- Chronically active disease (CAD): a SLEDAI-2K≥2 at least two out of the three annual visits; this indicates a disease that remains active for at least 8 months a year. (129)
- Relapsing-remitting disease (RRD): a SLEDAI-2K≥2 at one of the three annual visits. RRD describes fluctuating disease activity over the course of a year, with periods of activity interspersed with periods of quiescence. (129)

Classifying patients according to the course of their disease is useful to tailor therapy to clinical evolution.(128,129)

10. THERAPY

10.1. Therapeutic goals, EULAR recommendations and overarching principles

SLE treatment should preferably begin in the early stage of the disease to maximize the positive effect on long-term prognosis. The first aim of the therapy should be complete remission as quickly as possible. However, this isn't always an easy goal to achieve, therefore clinical remission or low disease activity should be pursued.

Inducing a rapid remission is critical to prolong long-term survival and prevent organ damage, moreover remission allows the improvement of patient's life quality.

Secondary goals, once remission is achieved, are to scale up and eventually discontinue glucocorticoids to prevent long-term side effects.

The 2023 EULAR overarching principles, suggest that SLE patients should be managed as much as possible by a multidisciplinary team, sharing therapeutic decisions with the patient, considering the need for patient education and individualization of therapy, while balancing it with cost efficacy. (132) Moreover, the general recommendations emphasize to assess disease activity and any potential organ involvement at each visit. (132).

On one hand, non-pharmacological interventions must be included in the treatment plan, such as smoking cessation, sun protection, have a regular and balanced diet, observe routinary physical activity and promote measures for bone health.

On the other hand, it's essential to tailor pharmacological interventions considering patient's features, comorbidities, current and risk of organ involvement, as well as patient's preferences when multiple comparable treatments are available. Moreover, drugs should be chosen according to the level of disease: mild moderate or severe. (132)

Finally, strict adherence to treatment is particularly desirable.

10.2. Hydroxychloroquine (HCQ)

Hydroxychloroquine is part of the antimalarial drugs group.(133) It enters and tends to accumulate in lysosomes, where it inhibits the degradation of externally or internally derived cargo in autolysosomes by increasing the pH to prevent the activity of lysosomal enzymes(134). Inhibition of lysosomal activity can prevent the presentation of autoantigens, mediated by MHC class II.(134) Furthermore, hydroxychloroquine inhibits GMP-AMP activity and thus prevents TLR signaling and cGAS-STING (cyclic GMP-AMP synthase-stimulator of interferon genes) signaling. Therefore, hydroxychloroquine reduces the production of pro-inflammatory cytokines, including type I interferons (see the chapter on IFN).(88,89)

Furthermore, HCQ interferes with immune activation at various cellular levels by inhibiting innate and adaptive immune processes. In APCs (antigen-presenting cells), hydroxychloroquine potentially interferes with the binding of TLR7 and TLR9 ligands and TLR signaling. (134) In APCs, such as pDCs (plasmacytoid dendritic cells) and B cells, this drug also suppresses antigen processing and subsequent MHC class II presentation to T cells, preventing the activation, differentiation, and expression of co-stimulatory molecules of T cells and also reducing cytokine production (such as IL-1, IL-6, and TNF) by both T cells and B cells.(134) Additionally, hydroxychloroquine has an antithrombotic effect due to its action of inhibiting platelet adhesion, aggregation, and activation, making it particularly important in patients with a prothrombotic tendency or APS (antiphospholipid syndrome)(60).

Hydroxychloroquine is one of the most used drugs in patients with SLE (132) and recommended by EULAR to be used in all patients unless contraindicated.

The recommended dose of hydroxychloroquine for all patients is 5 mg/kg unless contraindications regarding retinal toxicity arise; in patients with cutaneous involvement who have experienced retinal toxicity, quinacrine can be used as a substitute.(132)

10.3. Glucocorticoids (GC)

Glucocorticoids act through two mechanisms:

- A genomic mechanism, which is slow-acting, where GCs interfere with the genomic transcription of inflammatory molecules. (135) GCs bind to the cytosolic glucocorticoid receptor (cGR), the complex formed by this binding is translocated into the nucleus where it modulates gene expression. (135) The effect of GCs at the nuclear level is to repress genes that drive the inflammatory process (transrepression).(135) At the same time, GCs induce a process of transactivation resulting in gluconeogenesis, insulin resistance, skin atrophy, and inhibition of bone formation, all well-known adverse effects of GCs. This mechanism is more strongly stimulated by prednisone compared to dexamethasone or methylprednisolone.(132,135)
- The non-genomic mechanism is rapid (about 15 minutes) and acts by modulating inflammatory and immune cells through three molecular mechanisms independent of nuclear interactions.(135) Firstly, the GC-cGR complex directly blocks the activation of phospholipase A2 and thus the production of arachidonic acid. Secondly, activation of the membrane-bound glucocorticoid receptor reduces lymphocyte activity. Finally, nonspecific interactions with the cellular membranes of immune cells result in the inhibition of ATP production and thereby reduce cellular activity. This mechanism is particularly sensitive to methylprednisolone and dexamethasone, which have non-genomic effects up to five times more potent than genomic ones. For this reason, these two drugs are more frequently used in acute phases.(6,135)

According to recent EULAR recommendations, corticosteroids should be used as a bridge therapy in systemic lupus erythematosus (SLE), similar to what is suggested for rheumatoid arthritis. In SLE, corticosteroid treatment should be at the lowest possible dose for the shortest possible period. The optimal target is to achieve lasting remission without corticosteroids, but if necessary, the aim should be the lowest acceptable dose of 5 mg/day.(132)

Finally, in patients with moderate to severe disease, where severe flares occur, intravenous pulses of methylprednisolone at doses ranging from 125 to 1000 mg for 1-3 days may be considered. (132,135)

In general, if necessary, the dose of corticosteroids should be chosen based on the severity of organ involvement and then tapered down to a maintenance dose that should be ≤ 5 mg/day of prednisone (or equivalent glucocorticoids) and then, when possible withdrawn. (132)

These corticosteroid dose limits have been imposed because over the years, multiple side effects of corticosteroids have been observed. Therefore, the goal is to prescribe the minimum dose for a significant therapeutic effect or to withdraw GCs which is the optimal target.(132)

10.4. Immunosuppressive drugs (ISDs)

10.4.1. Mycophenolate mofetil (MMF)

Mycophenolate mofetil is obtained from the extraction of mycophenolic acid.(136) Its activity consists in inhibiting nucleic acid synthesis.(67) In B and T cells, mycophenolate inhibits de novo synthesis of guanine and prevents its incorporation into DNA, thereby inducing cell cycle arrest in the S phase, moreover this leads to the inhibition of lymphocyte proliferation and the apoptosis of T lymphocytes.(136,137)

10.4.2. Azathioprine (AZA)

Azathioprine is pro-drug of 6-mercaptopurine (imidazole derivative) synthesized in 1957. (60,136)

6-MP is inactive but acts as a purine antagonist, requiring cellular uptake and intracellular anabolism to thioguanine nucleotides (TGN) for immunosuppression.(136)

TGN and other metabolites (e.g., 6-methyl-mercaptopurine ribonucleotides) inhibit de novo purine synthesis and the interconversion of purine nucleotides.(138)

TGN is also incorporated into nucleic acids, contributing to the drug's immunosuppressive effects. Other potential mechanisms of azathioprine include the

inhibition of many nucleic acid biosynthesis pathways, preventing the proliferation of cells involved in initiating and amplifying the immune response. Due to these mechanisms, its effect is more pronounced in replicating lymphocytes.(138)
Because of these mechanisms, the therapeutic effect of azathioprine may only become evident after several weeks or months of treatment. The advantage of this drug is its low cost, good tolerability, and it is the recommended drug during pregnancy.(136)

10.4.3. Methotrexate (MTX)

Methotrexate belongs to the class of antimetabolites and possesses a structure akin to folic acid.(139) This similarity allows it to competitively inhibit dihydrofolate reductase, preventing the reduction of dihydrofolate to tetrahydrofolate, a necessary step in the DNA synthesis process and cell proliferation.(139,140) Generally, actively proliferating tissues are more sensitive to the action of methotrexate. It inhibits DNA synthesis and increases the release of adenosine, which has anti-inflammatory properties.(140)

10.4.4. Cyclophosphamide (CYC)

Cyclophosphamide is a pro-drug, nitrogen mustard which requires oxidation by the cytochrome P450 (CYP) system. (136) Once metabolized, CYC binds to DNA, causing the addition of an alkyl group to guanine at the nitrogen 7 position of the imidazole ring. (136) It also induces the formation of covalent bonds and the breaking of the DNA double strand, and finally inhibits DNA replication, leading to cell death, both in active and quiescent lymphocytes. (136,137) Particular attention must be paid to the further metabolism of CYC, which produces acrolein, a compound that can cause hemorrhagic cystitis.(6,136) In certain situations, such as when there is no valid response to hydroxychloroquine and/or GC therapy, or when a patient has experienced improvement but cannot reduce corticosteroid dosage below 5 mg/day, it is recommended to add immunosuppressive drugs. In cases where the disease presents as organ-threatening or life-threatening, the use of cyclophosphamide should be considered.(132)

10.4.5. Calcineurin inhibitors (CNIs)

Cyclosporine A, tacrolimus, and voclosporin are calcineurin inhibitors (CNIs).(136) They all have a similar mechanism of action. Cyclosporine A was the first to be used in SLE and is capable of inhibiting T lymphocyte proliferation, as well as suppressing the expression or activation of pro-inflammatory cytokines.

Calcineurin inhibitor molecules bind to cyclophilin A in T cells. This binding inhibits the action of calcineurin on NFAT (141) to prevents Th1 cytokines production. This also results in inhibition of growth factors for effector and memory cells.(142)

Voclosporin is a new CNI, approved in 2021 by the FDA and in 2022 by the EMA.(142) It is therefore an immunosuppressive drug approved for the treatment of lupus nephritis, as an add-on to other immunosuppressants such as mycophenolate and low-dose GCs.(142) The primary goal in lupus nephritis is to preserve renal function, to promote the patient's quality of life, and to reduce mortality.(132)

In kidneys calcineurin dephosphorylates synaptopodin, which is then metabolized by a lysosomal protease. This results in the destabilization of podocytes and increase of proteinuria.(141,142) Voclosporin, by inhibiting this dephosphorylation pathway, promotes the stabilization of the actin cytoskeleton in podocytes and thus reduces proteinuria. (141,142)

10.5. Biologics

There are three types of biologics included in the treatment recommendations for SLE patients: belimumab, anifrolumab, recently included in 2023 guidelines, and rituximab(132).

10.5.1. Anifrolumab (ANI)

SRI4 was considered effective as primary endpoint until anifrolumab (anti-type I interferon receptor antibody) failed phase III in clinical trials using SRI4 (92). SRI4 was then substituted with BICLA successfully. (92)

Anifrolumab is a humanized IgG1k monoclonal antibody which binds with high affinity and specificity to IFNAR1, inhibiting the formation of the IFN/IFNAR complex and subsequent gene transcription (147,148). Anifrolumab antagonizes the

receptor responsible for cellular signaling induced by IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω. (149)

Anifrolumab has been shown to correct defects in both the innate and adaptive immune systems in SLE patients. Since approximately 60-80% of adult SLE patients have elevated levels of type-1 IFN inducible genes, in these patients, especially those with a high type-1 IFN gene signature status, anifrolumab has normalized protein expression, reversed cytopenias, and normalized immune cell populations.(149)

TULIP-1 trial was the first phase III study of an anti-IFNAR antibody for SLE treatment, unfortunately it didn't achieve the primary efficacy endpoint (SRI). (150) However, anifrolumab showed clinical benefits through secondary endpoints such as BICLA response, GCs use reduction and organ-specific improvements (skin and joints). Anifrolumab was well-tolerated with an acceptable safety profile.(150)

TULIP-2 trial preserved a similar design and proposed BICLA as the primary endpoint.(151) This study provided further evidence on IFNAR blockade efficacy in moderately to severely active SLE.(147,148,151)

Furthermore, TULIP-2 demonstrated the efficacy of anifrolumab 300 mg through a range of clinically significant endpoints: the BICLA response rate at week 52, with treatment differences exceeding 16% compared to placebo, improvement in CLASI score, and reduction of flares (151). Anifrolumab was successful in reducing flares and disease activity in 8-12 weeks, maintaining improvements throughout the 52-week period.(151)

Additionally, anifrolumab was identified as a steroid-sparing treatment, which will potentially reduce the cumulative risk of long-term organ damage.(152) Finally, an eminent improvement in skin manifestations was found. (151)To date, there are still no real life studies for anifrolumab, however it seems to be a new pharmacological weapon which will be implemented in the treatment of SLE patients with skin and joint manifestations.(147,151,152)

10.5.2. Rituximab (RTX)

Rituximab is a monoclonal antibody targeting CD20, which is a member of the family of integral membrane proteins, expressed in B cells.(134) CD20 regulates the cell cycle and B cell differentiation. RTX, once bound to CD20, through the Fc portion, it

induces complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), and antibody-dependent cellular cytotoxicity (ADCC) (134). RTX may be used only in refractory systemic lupus erythematosus (SLE) in patients with organ-threatening or life-threatening disease (130).

One Italian real-life study explored cutaneous manifestations response to RTX (143). This study was based on a multicentre and observational cohort of adult patients with SLE, refractory to standard therapy and treated with at least one course of RTX.(143) It was designed to test efficacy and safety of off-label use of rituximab in refractory lupus. Response to RTX in terms of ECLAM. Cutaneous patients were 11 and were divided by subtypes. The first course of RTX treatment induced complete response in 8 cutaneous patients (72,7%), partial response in 2 cutaneous patients (18,2%) and 1 non-responder patient, who had vasculitis. However, in some cases, concurrent aggressive immunosuppressive therapy, particularly high-dose corticosteroids, may have confounded and masked the efficacy of rituximab.(143) To conclude, RTX showed a good efficacy in the treatment of active, refractory renal and extra-renal SLE. Safety profile was also good; however, in case of retreatment, a higher incidence of AEs, especially infusion reactions and infections should be expected.(143)

10.5.3. Belimumab (BEL)

1. **Characteristics and mechanism of action**

   Belimumab is a monoclonal antibody which targets and inhibits soluble BLyS (also known as BAFF, B-cell activating factor) (4,122,136).

   Treatment with belimumab has been observed to have a rapid effect on naive B cells at early stages of differentiation. In contrast, B cells at later stages (plasma cells and memory B cells) have a late response or no response at all (4,120).

2. **Randomized Controlled Trials: Efficacy**

   In 2003, belimumab was tested for the first time in mouse models (4). The phase I study was multicenter (20 sites), randomized, double-blind, placebo-controlled, dose-escalation of belimumab in patients with SLE.(144) Patients received belimumab 1.0, 4.0, 10, or 20 mg/kg or placebo administered intravenously over at least 2 hours. Intravenous (IV) belimumab revealed a linear pharmacokinetics over a dosage ranging from 1 to 20 mg/kg, small volume distribution, slow

clearance and a long terminal elimination half-life (8.5 to 14.1 days).(144) Furthermore, concomitant use of immunosuppressants, HCQ and GCs had no significant effect on belimumab concentration. (144)

Phase II analyses proved no difference in terms of SELENA-SLEDAI 4-point decrease between the belimumab arm (testing 1mg/kg, 4mg/kg, 10mg/kg) and the placebo arm. However, post-hoc analyses revealed that patients treated with belimumab and serologically active at T0 showed an improvement in SELENA-SLEDAI at week 52 compared to placebo.

Subsequently in the phase III studies (BLISS-52 and BLISS-76), SRI4 became the primary endpoint (see clinimetrics chapter) in order to demonstrate improvement using a broader indicator.(4,120,122)

On one hand, BLISS-52 enrolled 865 SLE patients from Latin America, Asia-Pacific, and Eastern Europe and followed them for 52 weeks. (122) Patients were randomized to receive treatment with belimumab 1mg/kg or 10mg/kg plus SOC (Standard Of Care) or placebo plus SOC. (122)

BLISS-76 enrolled 819 patients for 76 weeks adopting the same scheme of treatment used in BLISS-52. (120)

Both in BLISS-52 and BLISS-76, patients with neuro-lupus and severe lupus nephritis were excluded.(4) Furthermore, in both studies a better SRI4 response rate was achieved in the belimumab-treated group compared to the control arm. Post-hoc analyses revealed that belimumab plus SOC was more effective in patients with high disease activity (SELENA-SLEDAI $\geq$ 10), high ds-DNA, and low complement, with cutaneous and articular manifestations and in patients who had baseline corticosteroid treatment > 7.5 mg.(4,122)

The positive results of post-hoc analyses on the effect of reducing proteinuria led to BLISS-LN, a phase 3 study in which the primary endpoint was met; resulting in the approval of belimumab for lupus nephritis.(145)

Other studies compared subcutaneous and intravenous administration, finding them to be nearly equivalent in achieving primary endpoint (SRI4) and in maintaining belimumab's efficacy.(146)

Finally, the EMBRACE study showed a positive trend in the use of belimumab in African American descendents patients. However, the primary outcome wasn’t

achieved in these patients (147). This trial was necessary because these patients were underrepresented in BLISS-52 and BLISS-76.(4,147)

Finally, in the pivotal studies, the efficacy of belimumab 10mg/kg versus placebo was proven with both the achievement of the SRI4 response endpoint at week 52 and at week 76. The SRI4 criterion, which was more stringent than SELENA - SLEDAI alone, achieved good reliability as a complex indicator.(4,120,122) In the majority of belimumab arm there was a reduction of SELENA-SLEDAI ≥ 4 points, as well as an improvement in PGA, furthermore other secondary efficacy endpoints such as corticosteroid dose reduction was achieved between week 40 and 52.(122)

3. **Randomized Controlled Trials: Safety**

Regarding belimumab's safety pivotal studies claim that adverse events, laboratory abnormalities as well as serious and/or severe AEs (including infections, malignancies, and deaths) was similar across groups. Infusion reactions were slightly higher in patients treated with belimumab than in controls. Hypersensitivity reactions are possible, as reported in 4 out of 819 patients, including 2 allergic reactions (however, seemingly not directly related to belimumab) and 2 cases of angioedema (considered belimumab-related). All reactions were resolved with antihistamines and/or prednisone on the day of infusion. The most frequent adverse events were infections, but severe infections rate was low (reporting cellulitis, pneumonia and urinary tract infections as most frequent).(4,120,122,146)

4. **Real-world studies (OBSErve, Berliss, BeRLiSS-LN, and Berliss-JS)**

In addition, even in post-marketing studies efficacy was proved. Among the first post-marketing studies the OBSErve (evaluation of use of belimumab in clinical practice Settings) studies are remarkable.(148–153) They were real-life, multinational cohort studies, sponsored by industry, which had the aim to evaluate efficacy and safety in the real-life setting. The results stated that patients, treated with belimumab, experienced clinical and serological improvement, accompanied by a GC spairing effect (148–153). Also independent real-life studies have shown that belimumab reduces the number of flares, slows the progression of disease damage and has a GC sparing effect.(154) It also confirms the reduction of: ds-

DNA, complement normalisation and long-term maintenance of the effects obtained. (4)

Among these independent studies, BeRLiSS (Belimumab in Real-Life Setting Study) was the first and the most significant in Italy. It was a retrospective study analysing data generated by 24 italian centres and prospectively collected from 2013 to 2019.

BeRLiSS confirmed in a real-life context belimumab's efficacy and safety. Moreover, it studied the rate of remission achievement and low disease activity of belimumab in the largest European nationwide cohort of SLE patients followed prospectively in a real-life setting. (155)

It was found patients with higher disease activity at baseline (SLEDAI-2K score of ≥10) were more likely to achieve an SRI-4 response at different time points, but were less likely to achieve cumulative remission. (155) This because it requires a longer time to flatten a high clinical SLEDAI score to ≤2 or 0 (which represent respectively low disease activity and remission).

Patients with early SLE treated with belimumab responded earlier to treatment and continue to respond better in the long term, while patients with long-standing disease at baseline either have a delayed response (around 1 year, when the SRI-4 response difference between the groups is not significant) or, in the case of no response at 1 year, were significantly less likely to respond to belimumab therapy in the long term. Interestingly, the greatest achievement of remission, low disease activity, and SRI-4 response rates was observed within the first 12 months of treatment. Considering these outputs, authors suggested 12 months as an appropriate time window to assess patients for response.

Moreover, BeRLiSS established that the lower the baseline damage, the greater the probability of achieving remission over the course of the follow-up, in patients treated with belimumab. Furthermore, damage accrual with belimumab treatment didn't increase significantly in patients with a baseline SDI score of 0 at 12, 24, and 36 months.

Additionally, discontinuation due to ineffectiveness of belimumab mainly occurs when the patient has experienced prior flare-ups before starting belimumab (155).

BeRLiSS also showed that organ manifestations which respond better to belimumab include arthritis and skin rashes, especially in the acute stage of disease(155) confirming results from two phase III trials which stated that belimumab treatment improved overall SLE disease activity in the most common musculoskeletal and mucocutaneous organ domains (156). Conversely, patients with “rhupus” syndrome were unlikely to respond to belimumab, which led frequently to discontinuation due to inefficacy.

Overall, DAS28 scores and CLASI activity was significantly improved in BeRLiSS cohort.

However, only musculoskeletal involvement emerged as a predictor of SRI-4 response at 12 months, whereas baseline skin involvement reduced the response rate at 6 months, electing skin involvement as a predictor of delayed response (155). However, in BeRliSS, skin involvement was positively associated with low disease activity, suggesting that skin manifestations require a longer time to resolve and occur during a window of time during which the CLASI and SLEDAI-2K indices may fail to capture clinically relevant changes occurring before, or instead of, a complete resolution.

Finally, BeRLiSS stated that early use of belimumab leads to favourable outcomes, whereas its use in patients with a long and rich history of disease flares or chronic active use of belimumab doesn’t give certain efficacy (4,155).

BeRLiSS-JS and BeRLiSS-LN were then proposed, the former with a focus on joint and skin manifestations, the latter on lupus nephritis.

BeRLiSS-JS confirmed in clinical practice data from randomized control trials showing the high effectiveness of belimumab in patients with joint and skin involvement.

BeRLiSS-JS observed remission in SLE patients according to different disease activity indices, including SLEDAI-2K, DAS28, and CLASI. Remission so described was achieved in one third of refractory SLE patients. In addition, almost half of the patients could achieve LDA after 12 months of belimumab therapy. This study also showed the glucocorticoid-sparing effect of belimumab, because a decrease in daily PDN dose intake was observed. Furthermore, a consistent proportion of patients could withdraw PDN during the follow-up.

Use of belimumab in the early stage of the disease was associated with a better SRI-4 response and damage prevention, a short disease duration and a lower DAS28 or CLASI at baseline predicted DAS28 and CLASI remission or LDA during the follow-up, suggesting that the earlier the use of belimumab, the better the outcome, regardless of the organ-specific activity score used to quantify organ-specific activity. Another critical aspect was the choice of outcome measures. A too-stringent response criteria might have contributed to several RCT failures. For instance, BeRLiSS study affirms that, in patients with skin involvement and high disease activity (CLASI > 10), where the achievement of remission is more difficult, CLASI-50 could be a reasonable and clinically meaningful outcome, especially in the short term. In patients with skin involvement, the lack of CLASI remission early during belimumab treatment did not prevent its achievement later during the follow-up, suggesting that 6 months shouldn't be enough to test for belimumab efficacy. BeRLiSS-JS suggested one year as the adequate time span of belimumab therapy needed to thoughtfully evaluate its efficacy. However, patients showing an earlier and higher improvement have a greater probability of achieving remission.

To conclude, BeRLiSS-JS demonstrated efficacy in inducing remission in one third of patients with refractory disease, according to disease activity indices, such as SLEDAI, DAS28 and CLASI-A. (157) Skin remission was achieved in 36.7% of patients between 6 and 36 months, while LDA (low disease activity) was observed in 32.4% to 69% of patients between 6 and 36 months.(157) There was also a significant reduction after 12 and 36 months in patient with CLASI-A > 10 at baseline. Among these patients, none had a CLASI-A > 10 at the end of the study. In addition, the proportion of patients treated with PDN ≤ 5 mg/day and without GCs increased significantly, thus proving in real life GCs sparing effect. (4,157)

BeRLiSS-LN

The aim of the study was to prove belimumab's efficacy on renal manifestation of the disease, in real-life treated patients. (158)

Primary efficacy renal response (PERR), defined as proteinuria ≤ 0.7 g/24 h, eGFR≥60 ml/min/1.73 m^2 without rescue therapy, was considered as primary

outcome. Whereas complete renal response (CRR; proteinuria <0.5 g/24 h, eGFR≥90 ml/min/1.73 m^2) was considered as secondary outcome.(158)

The results show that 66.1% of patients achieved partial renal response (PERR) and 37.3% complete response (CRR) at 24 months. (158) The mean time to achieve PERR was less than 12 months. However, if compared with BLISS-LN, in BeRLiSS-LN cohort belimumab treatment started after initial therapy for LN and at lower baseline levels of proteinuria. (158) In addition, elevated baseline proteinuria and creatinine, smoking and hypertension were associated with poor renal response. Nevertheless, rate of renal flare-ups after treatment initiation with belimumab was low, confirming the effect of reducing flare-ups observed in the BLISS trials and reconfirming efficacy on lupus nephritis. (4,158)

10.6. EULAR recommendations in non-renal SLE

In SLE with non-renal involvement, the lines of treatment are divided according to disease severity.(132)

	1st line	2nd line
	HCQ in all patients (unless contraindicated)	
	GC per os o i.v. if needed	
Mild		MTX, AZA, MMF, BEL, ANI
Moderate	MTX, AZA, MMF, BEL, ANI, CNI	MMF, BEL, ANI, CNI
Severe	MMF, BELI, ANI, CYC, RTX	CYC, RTX

Table XIII: inspired by (60,132)*. Treatment of non-renal SLE. Cells of the table show drugs which aren't in order of preference thus are equivalent options. Anifrolumab and belimumab, recommended as first line therapy in severe disease, refers to patients with extensive disease from joint and skin, but exclude non-renal SLE patient with major organ involvement* (132)*. ANI=anifrolumab; AZA=azathioprine; BEL=belimumab; CNI=calcineurin inhibitor; CYC=cyclophosphamide; GC=glucocorticoids; HCQ=hydroxychloroquine; MMF=mycophenolate mofetil; MTX=methotrexate; RTX=rituximab.*

10.6.1. Recommendations for skin disease

Skin disease therapy includes topical and systemic treatment. (60,132) EULAR recommendations for the patient with active CLE suggest as first line therapy: topical agents, antimalarials (of which HCQ is the antimalarial of choice) and/or glucocorticoids.(132)

Commonly used topical treatments for all forms of cutaneous lupus (acute, subacute, and chronic) include tacrolimus, R-salbutamol, pimecrolimus, clobetasol, betamethasone and photoprotection.(60)

In cases of severe and extensive skin involvement, treatment is based on systemic pharmacological options. (1)

Antimalarials, are the first-line treatment, and HCQ is preferred at the maximum daily dose of 5 mg/kg (hydroxychloroquine); as mentioned earlier, patients on antimalarials should be followed for the risk of ocular toxicity. (132)

Indications for systemic GC include very acute and severe skin lesions, even in addition to antimalarials, considering the slow effect of steroids.(1,132) The standard oral dose of prednisone is 0.5 mg/kg, while 3-day pulses of intravenous methylprednisolone are available during exacerbations.(135)

First-line therapies fail in about 40% of patients; in these cases, patients may benefit from a wide range of second-line systemic treatments (132). Anifrolumab, belimumab, methotrexate (MTX) or mycophenolate mofetil are part of the armamentarium for refractory skin disease.(132) As seen previously both anifrolumab and belimumab have shown efficacy in mucocutaneous manifestations of SLE and both have few side effects when compared with other immunosuppressive drugs. (154,157,159) However in the recently updated EULAR 2023 guidelines Anifrolumab showed 1a class of recommendation, level A, while belimumab exhibited 1a, but level B. (132)

Drugs that may be considered as second- or third-line options include dapsone, retinoids, CNI, AZA, CYC, and RTX, ideally in collaboration with dermatologists experienced in treating CLE. (132) Finally, thalidomide and lenalidomide, are effective in various subtypes of cutaneous lupus, however, both should be reserved for patients who have failed with multiple previous agents and with the utmost caution in women of reproductive age.(132)

AIM OF THE THESIS

The efficacy of belimumab in SLE has been extensively proven, not only by considering randomized controlled trials, but also confirmed in real-life observational studies. However, there is only one post-hoc analysis of a phase III studies BLISS52/BLISS76 in which patients were stratified by type of skin manifestations (156). In that case, patients were sorted by several cutaneous manifestations as follows: maculopapular eruption (mild), alopecia (mild), alopecia (severe, active), active discoid lesions, periungual erythema, malar erythema, small mucosal ulceration. Results revealed that belimumab was associated with significant improvements in maculopapular eruption (mild), alopecia (both in mild and in severe, active) and active discoid lesions if compared to placebo (156). However, to date, no studies, carried out in real life setting, had evaluated patients by sorting them into clinical cutaneous subtypes.

Thus, the aim of the thesis was to observe if different subtypes of cutaneous SLE, including ACLE, SCLE, CCLE, vasculitis, livedo and alopecia/lupus hair, would have influenced the efficacy of belimumab.

CLASI-A and CLASI-D were used to assess the response to belimumab in patients with different subtypes of cutaneous involvement.

The primary outcome was to identify, if existent, better responder to belimumab among patients with specific and non-specific cutaneous manifestation.

The secondary outcome consisted in understanding the real-life response times to belimumab in terms of CLASI decrease and remission (defined as CLASI=0) among the same cohort of patients with specific and non-specific skin manifestations. GCs dose reduction was also investigated.

MATERIALS AND METHODS

1. BeRLiSS-Skin

The BeRLiSS-Skin study was designed as retrospective and observational, enrolled adult SLE patients, treated in Italy in 14 reference centres from 2013 to May 2024, homogeneous for data collection and application of 2019 and then 2023 EULAR guidelines for treatment.

1.1. Inclusion and exclusion criteria

Inclusion criteria were the follows:

- Fulfilment of the American College of Rheumatology (ACR) 1982 revised criteria for SLE (64) or the European League Against Rheumatism EULAR/ACR classification criteria for SLE (70);
- Adult patients treated with belimumab for at least 6 months, considering both IV and SC route of administration; using IV 10 mg/kg (on days 1, 14, and 28, and then every 28 days) or SC 200 mg/week. The inclusion of both SC and IV is endorsed by the study BLISS-SC which compared the effectiveness of oral administration with intravenous administration and concluded that they are equally effective (120).
- Patients with active disease, defined by a clinical SLEDAI > 0, and cutaneous active disease, defined by CLASI-A > 0, at belimumab initiation.
- SLE patients treated between January 1, 2013 and May 31, 2024.

Exclusion criteria were the follows:

- Insufficient data entry
- Age<16y

Management of patients in this study did not interfere with clinical practice.

Patients were stratified at the outset on the basis of the skin phenotype in:

- Specific
  - ACLE
  - SCLE
  - CCLE

- Non-specific
  - Cutaneous vasculitis
  - Alopecia/lupus hair
  - Livedo reticularis

Standard of care was defined according to the 2023 EULAR recommendations for the management of SLE (132).
The study was approved by the University of Padua Ethics Committee (3806/AO/16) and carried out according to Helsinki Declaration.

1.2. Variables considered in this study

The general data collected consisted of: date of birth, sex, date of diagnosis, age at diagnosis, age at the onset of belimumab, duration of SLE pre-belimumab, pregnancies pre-belimumab, miscarriages pre-belimumab, type of disease course before starting belimumab (categorizing it in chronic active or relapsing remitting), antibody profile (ANA, anti-dsDNA, anti-Sm, anti-SSA, anti-SSB, anti-U1RNP, anti-P ribosomal, antiphospholipids, APS, overlap with other autoimmune diseases). Previous therapies were also included: HCQ, MTX, AZA, CYC, cyclosporine A, MMF, RTX, others if present. Previous organ involvement was embedded: arthritis, cutaneous, kidney with the date and class of the last biopsy if available, neurological, serositis, hematological, others). Comorbidities such as diabetes, hypertension, dyslipidemia, neoplasms, vasculopathy, ischemic heart disease, osteoporosis, smoking, menopause < 40 years were also collected and reported if present. Finally, information about concomitant therapy at the start of belimumab (including MMF, MTX, AZA, cyclosporine A, HCQ, CQ) was collected.
Clinical and laboratory variables were gathered at baseline and every 6 months as follows: daily prednisone intake, levels of anti-dsDNA, complete blood cell count, levels of C3 and C4, 24h proteinuria, sediment, creatinine.
Clinimetric data consisted in: global activity indices (SLEDAI-2K, PGA), organ-specific activity indices, particularly emphasizing cutaneous manifestations using

CLASI-A and CLASI-D and finally considering responder and damage indices, respectively SRI4 and SLICC-DI.

Variables were all examined at baseline and after 6, 12, 18, 24, 30, 36 months since the onset of belimumab.

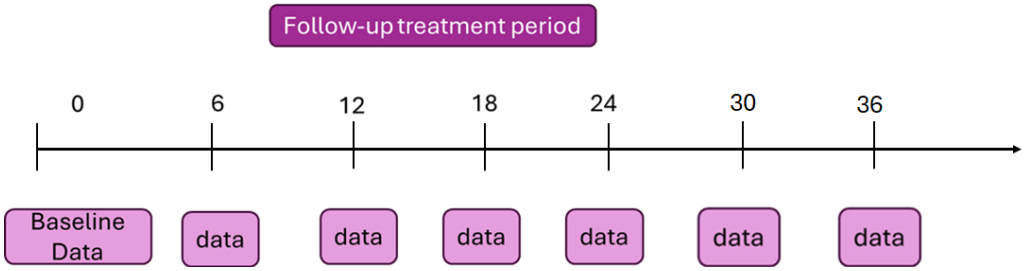


Figure 13: follow-up treatment period

1.3. Outcome Measures

BeRLiSS-Skin study analyzed: the variation of CLASI-A and CLASI-D scores, the achievement of CLASI-A remission, defined as CLASI-A=0; and daily prednisone intake, all examined at baseline and after 6, 12, 18, 24, 30, 36 months since the onset of belimumab.

1.4. Discontinuation

Discontinuation was defined as an interruption of belimumab for more than 6 months.

Reasons for discontinuation were:

- Inadequate response/inefficacy, defined by physician judgment as the presence of flares and/or the persistence of moderate/high disease activity;
- Adverse events (AEs) and severe AEs, which were recorded at each clinical evaluation during the follow-up;
- Pregnancy and loss of follow-up/transfer.

1.5. Statistical Analysis

Parametric and non-parametric tests were used according to the data distribution displayed by each variable.

To perform comparisons between groups, χ^2-test was employed for categorical dicotomic data. Continuous data with non-parametric distribution were analyzed using Wilcoxon's rank sum test and Wilcoxon's test for paired data. To assess the variation over time of different variables, ANOVA test and Friedman's test with Bonferroni's correction were used for parametric and non-parametric data respectively. For comparisons between three or more groups, ANOVA for repeated measures was utilized for parametric data and one-way repeated measures analysis of variance by ranks through Friedman's test was utilized for non-parametric data. p-values less than 0.05 were considered significant.

RESULTS

1. General baseline data

A total of 443 patients were enrolled (F=394; 88.9%), with mean age at diagnosis 29.9±13.2 years and mean treatment duration 52.2±38.0 months. At belimumab initiation 242 patients (54,6%) had skin manifestations: 112 acute (25.3%), 54 subacute (12.1%) and 18 chronic cutaneous lupus (4.1%), 48 cutaneous vasculitis (10,8%), 23 livedo reticularis (5.2%), 79 alopecia/lupus hair (17.8%).

Demographic, clinical and serological features and concomitant treatment at baseline are reported in Table XIV.

Data collected	**Results**
Patients, N	443
Patients with cutaneous manifestation, N	242 (54,6%)
Female	394 (88,9%)
Male	49 (11,1%)
Age at SLE diagnosis, years, mean ±SD	29.9±13,2
Age at the first infusion years, mean ±SD	47,5±12,4
Disease duration at recruitment, years, mean ± SD	12,0±10,5
Relapsing-remitting, N	270
Chronic-active, N	171
Antibodies profile	
ANA, N (%)	441 (99,5%)
ANTI-DNA, N (%)	397 (89,6%)
ANTI-SM, N (%)	122 (27,7%)
ANTI-SSA, N (%)	199 (45,3%)
ANTI-SSB, N (%)	69 (15,7%)
ANTI-URNP, N (%)	139 (31,6%)
ANTI-P RIB, N (%)	30 (7%)
ANTI-FOSFOL, N (%)	141 (32,1%)

APS, N (%)	59 (13,5%)
Concomitant treatment (dosage) at onset of belimumab	
MMF (g) median± SD	2±0,75 (31,8%)
MTX (mg/week) median ± SD	11,3±5,6 (10,3%)
AZA (mg) median ± SD;	100±45 (18,6%)
CsA median ± SD	100±63 (7,4%)
HCQ (mg) median ± SD	300±82 (71,5%)
Previous therapy	
MMF; N (%)	201 (45,4%)
MTX; N (%)	196 (44,2%)
AZA; N (%)	194 (43,8%)
CsA; N (%)	115 (26%)
HCQ; N (%)	245 (55,3%)
CYF; N (%)	76 (17,2%)
RTX; N (%)	50 (11,3%)
Previous clinical manifestations	
Arthritis N (%)	388 (87%)
Cutaneous; N (%)	315 (71,3%)
Renal; N (%) Class; N: • 0 • II • III • IV • V • II-V • III-IV	166 (37,5%) 32 17 23 62 15 1 1
Neurologic; N (%)	57 (13,0%)
Serositis; N (%)	131 (29,6%)
Hematological; N (%)	235 (53,0%)

Baseline data	
PDN mg/die, mean ±SD	10,2 ± 8,6
C3 (mg/dl), mean ±SD	74,2 ± 23,2
C4 (mg/dl), mean ±SD	11,9 ± 7,7
GB (mmc), mean ±SD	5279 ± 2631
Lymphocytes (mmc), mean ±SD	1302 ± 671
Hb (g/dl), mean ±SD	12,1 ± 1,4
Platelets, mean ±SD	229107 ± 98583
24h proteinuria (g/die), mean ±SD	2,6 ± 31,8
eGFR, mean ±SD	90,2 ± 21,8
Fatigue (VAS 0-10), median ±SD	5 ± 2,7
SLEDAI-2K, mean ±SD	9 ± 4
PGA, mean ±SD	2,2 ± 1,5
SLICC-DI, mean ±SD	1 ± 1

Table XIV: data collected before starting belimumab therapy.

The table below regards the percentages of active manifestations at the initiation of belimumab. Note that skin manifestations were categorized by subtype.

Manifestations			**N° (%)**
Cutaneous	Overall		242 (52,1%)
	1. Specific	ACLE	112 (46,3%)
		SCLE	54 (22,3%)
		CCLE	18 (7,4%)
	2. Non-specific	Cutaneous vasculitis	48 (19,8%)
		Livedo reticularis	23 (9,5%)
		Alopecia/lupus hair	79 (32,6%)
Haematological			152 (34,3%)
Kidney			106 (23,9%)
Joint	Overall		272 (61,4%)

	NDNE	221 (81,3%)
	Jaccoud	30 (11,0%)
	Rhupus	21 (7,7%)
Serositis		43 (9,7%)
Constitutional		191 (43,2%)

Table XV: percentages of active manifestations at the initiation of belimumab, categorized by subtypes in skin and joint.

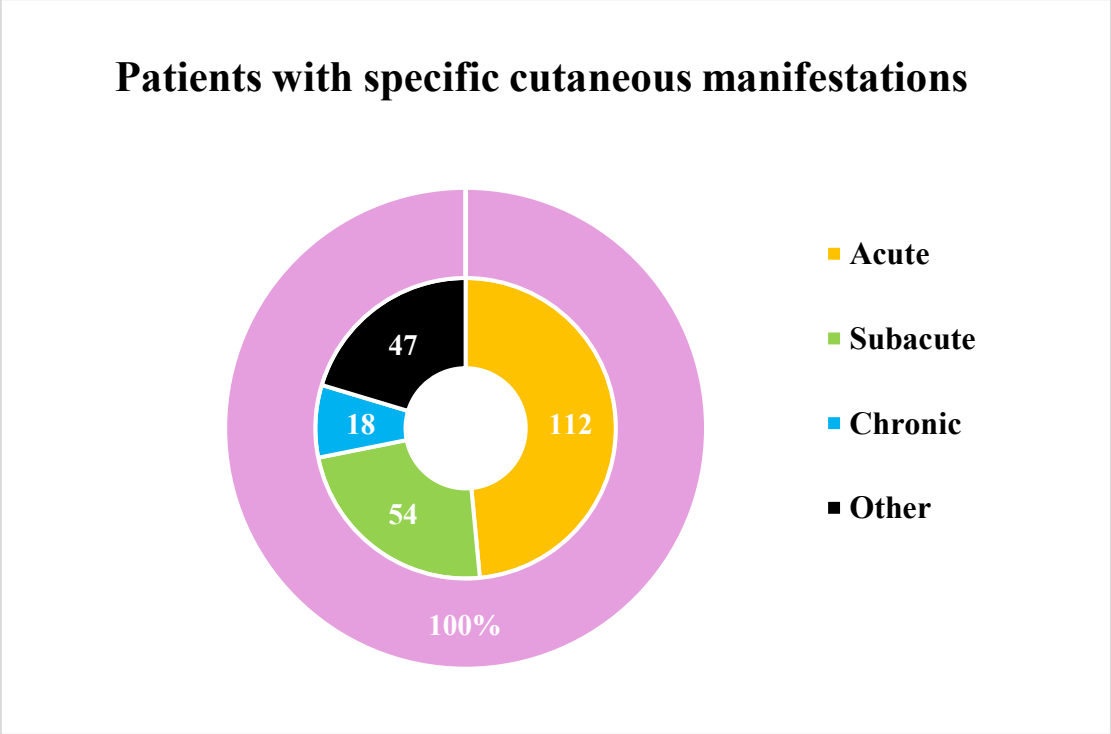


Figure 14: number of patients with specific cutaneous manifestation which led to belimumab treatment. Notably, 242 patients had cutaneous manifestations.

	Specific skin manifestations		Specific + Non-specific skin manifestations	
	Mean±SD	**Median (IQ range)**	**Mean±SD**	**Median (IQ range)**
CLASI-A	4±4	3 (1 – 6)	4±4	3 (1 – 6)
CLASI-D	1±1	0 (0 – 0)	1±1	0 (0 – 0)
SLEDAI-2K	10±4	10 (6 –12)	10±4	10 (6 – 12)
SLICC_DI	1±1	1 (0 – 1)	1±1	1 (0 – 1)
PDN mg/die	10,1±8,5	7,5 (5-12,5)	7,5±8,6	7,5 (5 – 12,5)
C3 (mg/dl)	73,8±25,9	72 (60 – 86,5)	71±25,7	71 (60 – 86)
C4 (mg/dl)	11,8±8,5	10 (6 – 15)	9±8,4	9 (6 – 15)
Fatigue (VAS 0-10)	5,0±2,7	5 (3 – 7)	5,0±2,7	5 (3 – 7)
PGA	2,19±1,5	2 (1,5 – 2,5)	2±1,6	2 (1,5 – 2,5)

Table XVI: data exposed considers patients with active cutaneous lupus, dividing data by considering only specific cutaneous manifestations or considering both specific and non-specific cutaneous manifestations together. IQ range= interquartile range; SD= standard deviation.

Summarizing for patients with active cutaneous SLE at baseline, the median CLASI-A score was 3±4 SD, and the CLASI-D score was 1±1; females accounted for 219 (90.5%), and males for 23 (9.5%); age at diagnosis was 28.2±12.8 years; age at the start of belimumab treatment was 46.1±11.70 years; thus, with an average disease duration before starting belimumab of 12.4±11.3 years. The disease course was relapsing-remitting in 143 patients (59.6%) and chronic-active in 96 patients (40.3%). Regarding antibody profile, ANA was positive in 99.6% of patients, anti-DNA in 91.3%, anti-Sm in 31.5%; anti-SSA in 46.8%; anti-SSB in 18.6%; anti-URNP in 37.0%; anti-P in 9.4%; anti-phospholipids in 27.9%; 26 patients (11.0%) had APS, while 10.9% had overlap with other autoimmune diseases.

Among patients with active cutaneous manifestations, 112 had ACLE, 54 had SCLE, and 18 had CCLE. At baseline, 48 patients had cutaneous vasculitis; 23 had livedo reticularis, and 79 had alopecia/lupus hair.

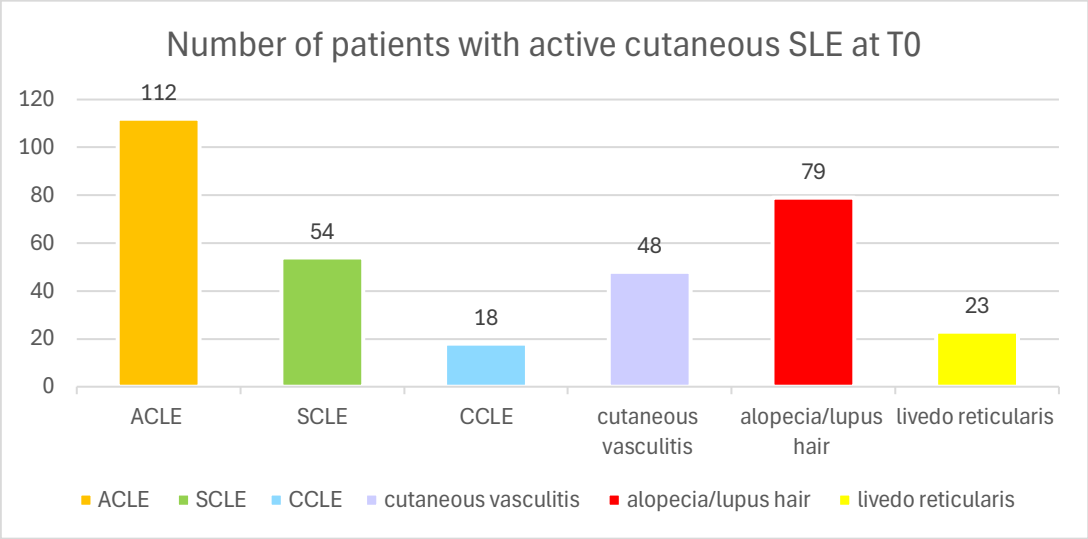


Figure 15: histogram representation of the number of patients with different subtypes of cutaneous manifestations, also considering in the count those patients who had more than one manifestation.

		N. valid	N. discontinuation		N valid	N. discontinuation		N.valid	N.discontinuation
T0	**Cutaneous oveall**	242	0	Acute	112	0	Cutaneous vasculitis	48	0
				Subacute	54	0	Livedo reticularis	23	0
				Chronic	18	0	Alopecia/lupus hair	79	0
T6		225	17	Acute	100	12	Cutaneous vasculitis	45	3
				Subacute	53	1	Livedo reticularis	21	2
				Chronic	16	2	Alopecia/lupus hair	73	6
T 12		203	39	Acute	90	22	Cutaneous vasculitis	38	10
				Subacute	47	7	Livedo reticularis	17	6
				Chronic	15	3	Alopecia/lupus hair	66	13
T 18		175	67	Acute	71	41	Cutaneous vasculitis	32	16
				Subacute	44	10	Livedo reticularis	14	9
				Chronic	12	6	Alopecia/lupus hair	55	24
T 24		156	86	Acute	63	49	Cutaneous vasculitis	31	17
				Subacute	39	15	Livedo reticularis	13	10
				Chronic	13	5	Alopecia/lupus hair	49	30
T30		134	108	Acute	55	57	Cutaneous vasculitis	27	21
				Subacute	33	21	Livedo reticularis	11	12
				Chronic	11	7	Alopecia/lupus hair	40	39
T36		108	134	Acute	48	64	Cutaneous vasculitis	24	24
				Subacute	29	25	Livedo reticularis	9	14
				Chronic	6	12	Alopecia/lupus hair	26	53

Table XVII: number of patients divided by: timing; subtype of cutaneous manifestations; and by number of analysable patients or number of patients who discontinued belimumab.

2. Cutaneous efficacy on specific subtypes

Cutaneous phenotype	**Acute**	**Subacute**	**Chronic**
CLASI-A at baseline (n=242)	3.0 (1.0-6.0)	5.0 (2.0-8.0)	6.0 (2.0-7.0)
Baseline vs 6 months	*p<0.001*	*p<0.001*	*p=0.297*
CLASI-A at 6 months (n=225)	1.0 (0.0-3.0)	2.0 (0.0-5.0)	2.0 (0.0-6.0)
6 vs 12 months	*p=0.416*	*p=0.215*	*p=0.005*
CLASI-A at 12 months (n=203)	0.0 (0.0-2.0)	0.0 (0.0-3.5)	2.0 (0.0-3.0)
12 vs 18 months	*p=0.321*	*p=0.821*	*p=1.000*
CLASI-A at 18 months (n=175)	0.0 (0.0-0.0)	0.0 (0.0-2.5)	1.0 (0.0-2.0)
18 vs 24 months	*p=0.682*	*p=0.496*	*p=0.655*
CLASI-A at 24 months (n=156)	0.0 (0.0-0.0)	0.0 (0.0-2.5)	1.0 (0.0-2.0)
24 vs 30 months	*p=0.825*	*p=0.850*	*p=0.357*
CLASI-A at 30 months (n=134)	0.0 (0.0-2.0)	0.0 (0.0-2.0)	2.0 (0.5-5.0)
30 vs 36 months	*p=1.000*	*p=1.000*	*p=0.707*
CLASI-A at 36 months (n=108)	0.0 (0.0-0.0)	0.0 (0.0-1.0)	2.0 (0.0-2.5)
Baseline vs 36 months	*p<0.001*	*p<0.001*	*p<0.001*

Table XVIII: Stratification of CLASI-A score variation for different skin phenotypes. CLASI-A scores are reported as median and interquartile range. P values were assessed by Friedman's test.

Below there's scatter plots showing the trend of CLASI-A in time regarding acute, subacute and chronic subtypes with their respective interquartile ranges.

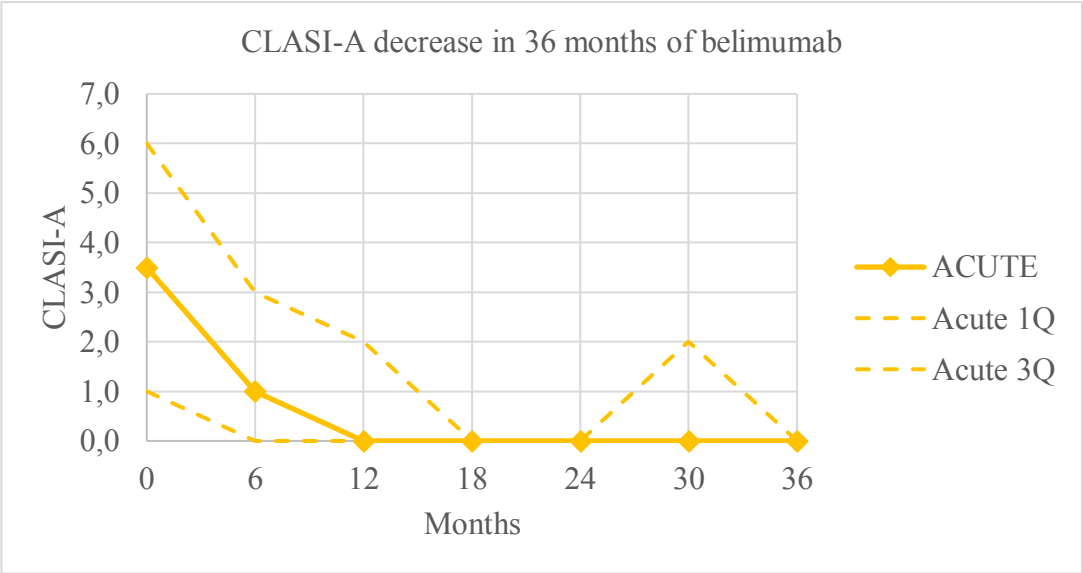


Figure 16: CLASI-A reduction in acute subtype of SLE, median and interquartile range.

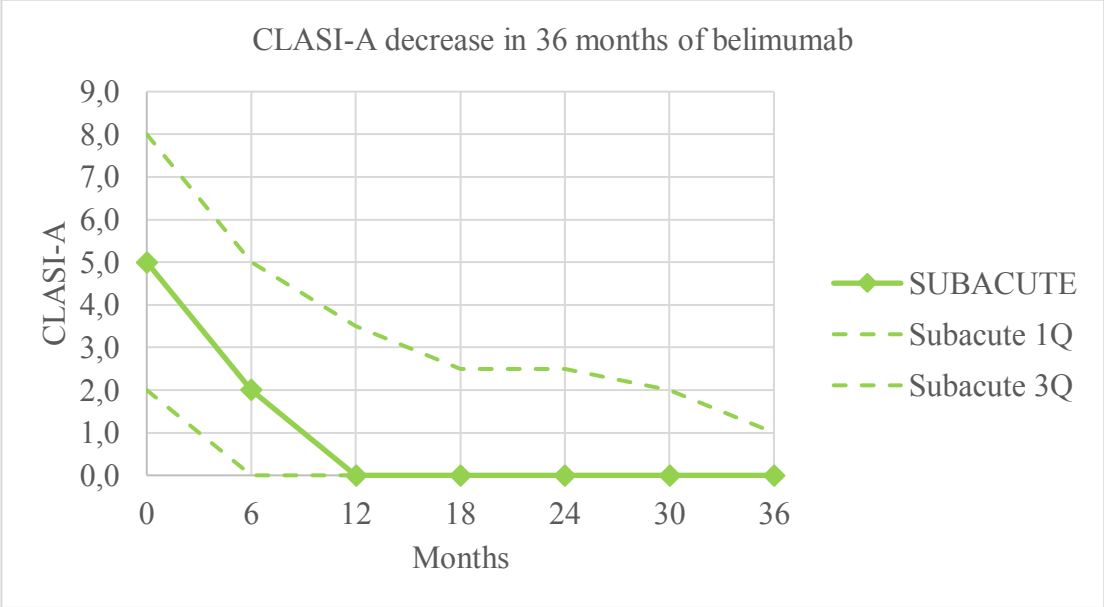


Figure 17: CLASI-A reduction in subacute subtype of SLE, median and interquartile range.

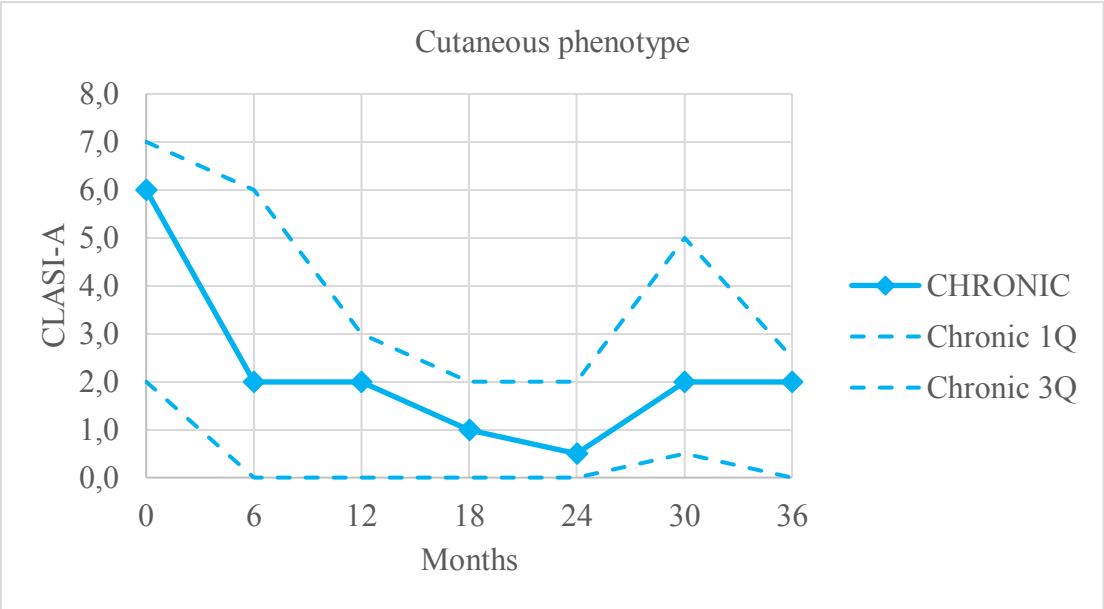


Figure 18: CLASI-A reduction in chronic subtype of SLE, median and interquartile range.

CLASI-A decreased significatively at 12, 24, and 36 months in all phenotypes if compared with baseline.

A statistically significant decrease in CLASI-A from baseline was observed as early as 6 months for the acute ($p<0.001$) and subacute phenotype ($p<0.001$), as late as 12 months for the chronic one (0-6months $p = 0.297$, 0-12 months $p=0.003$).

3. Cutaneous efficacy on non-specific subtypes

3.1. Cutaneous vasculitis

	Mean	Median	25% percentile	75% percentile	Number of patients	**p-value**
N of cases					14	
CLASI-A_0	2	2	0	3		
CLASI-A_12	1	0	0	1		
CLASI-A_24	1	0	0	0		
Variation of CLASI-A_0, CLASI-A_6, CLASI-A_12, CLASI-A_18, CLASI-A_24						0,092

CLASI-A_6 vs CLASI-A_0	0,264
CLASI-A_6 vs CLASI-A_12	1,00
CLASI-A_6 vs CLASI-A_18	1,00
CLASI-A_6 vs CLASI-A_24	1,00
CLASI-A_12 vs CLASI-A_0	0,264
CLASI-A_12 vs CLASI-A_18	1,00
CLASI-A_12 vs CLASI-A_24	1,00
CLASI-A_18 vs CLASI-A_0	0,264
CLASI-A_18 vs CLASI-A_24	1,00
CLASI-A_24 vs CLASI-A_0	0,264

Table XIX: general data about number of patients; mean, median, and interquartile range of CLASI-A. Analysis of variance by Friedman's 2-way ranks to correlated samples (with eventual correction of Bonferroni for multiple tests). No significance for vasculitis was found.

In the analysis, patients who had also specific manifestations were excluded, resulting in a decreased number of patients with isolated cutaneous vasculitis.

3.2. Livedo reticularis

	Mean	Median	25% percentile	75% percentile	Number of patients	p-value
N of cases					7	
CLASI-A_0	2	2	0	3		
CLASI-A_12	1	0	0	1		
CLASI-A_18	1	0	0	0		
Variation of CLASI-A_0, CLASI-A_6, CLASI-A_12, CLASI-A_18, CLASI-A_24						0,012
CLASI-A_6 vs CLASI-A_0						0,066
CLASI-A_6 vs CLASI-A_12						1,00
CLASI-A_6 vs CLASI-A_18						0,713
CLASI-A_12 vs CLASI-A_0						0,066
CLASI-A_12 vs CLASI-A_18						0,713

CLASI-A_18 vs CLASI-A_0	0,027

Table XX: general data about number of patients; mean, median, and interquartile range of CLASI-A. Analysis of variance by Friedman's 2-way ranks to correlated samples (with eventual correction of Bonferroni for multiple tests). Significance for livedo was found overall and at 18 months vs baseline.

In the analysis, patients who had also specific manifestations were excluded, resulting in a decreased number of patients with isolated livedo reticularis.

3.3. Lupus hair/alopecia

	Mean	Median	25% percentile	75% percentile	Number of patients	p-value
N of cases					16	
CLASI-A_0	2	2	0	3		
CLASI-A_12	1	0	0	1		
CLASI-A_24	1	0	0	0		
Variation of CLASI-A_0, CLASI-A_6, CLASI-A_12, CLASI-A_18, CLASI-A_24						0,043
CLASI-A_6 vs CLASI-A_0						0,205
CLASI-A_12 vs CLASI-A_6						0,74
CLASI-A_18 vs CLASI-A_6						0,499
CLASI-A_24 vs CLASI-A_6						0,499
CLASI-A_12 vs CLASI-A_0						0,108
CLASI-A_18 vs CLASI-A_12						0,735
CLASI-A_24 vs CLASI-A_12						0,735
CLASI-A_18 vs CLASI-A_0						0,052
CLASI-A_18 vs CLASI-A_24						1,00
CLASI-A_24 vs CLASI-A_0						0,052

Table XXI: general data about number of patients; mean, median, and interquartile range of CLASI-A. Analysis of variance by Friedman's 2-way ranks to correlated samples (with eventual correction of Bonferroni for multiple tests). No significance for alopecia/lupus hair was found, except of borderline result of overall p-value.

In the analysis, patients who had also specific manifestations were excluded, resulting in a decreased number of patients with isolated alopecia-lupus hair.

Decrese of CLASI-A was noted as late as 18 months for livedo reticularis (0-12 months p=0,066, 0-18 months p=0,027). No significant decrease in CLASI-A was found for the other non-specific skin manifestations of SLE.

4. CLASI-D variation

Variation of CLASI-D in all cutaneous subtypes	p-value
CLASI-D, CLASI-D_6, CLASI-D_12, CLASI-D_18, CLASI-D_24, CLASI-D_30 e CLASI-D_36	,089
CLASI-D- CLASI-D_6	,941
CLASI-D_6 vs CLASI-D_12	,782
CLASI-D_6 vs CLASI-D_18	,428
CLASI-D_6 vs CLASI-D_24	,782
CLASI-D_6 vs CLASI-D_30	,606
CLASI-D_6 vs CLASI-D_36	,606
CLASI-D_12 vs CLASI-D	,839
CLASI-D_12 vs CLASI-D_18	,606
CLASI-D_12 vs CLASI-D_24	1,000
CLASI-D_12 vs CLASI-D_30	,811
CLASI-D_12 vs CLASI-D_36	,811
CLASI-D_18 vs CLASI-D	,473
CLASI-D_18 vs CLASI-D_24	,606
CLASI-D_18 vs CLASI-D_30	,782

CLASI-D_18 vs CLASI-D_36	,782
CLASI-D_24 vs CLASI-D	,839
CLASI-D_24 vs CLASI-D_30	,811
CLASI-D_24 vs CLASI-D_36	,811
CLASI-D_30-CLASI-D	,659
CLASI-D_30-CLASI-D_36	1,000
CLASI-D_36-CLASI-D	,659

Table XXII: Friedman's two-way rank analysis of variance for related samples with Bonferroni correction.

	p-value					
	Acute	Subacute	Chronic	Cutaneous vasculitis	Livedo reticularis	Alopecia/ lupus hair
CLASI-D_36 vs CLASI-D	0,508	1,000	0,770	1,000	1,000	1,000

Table XXIII: Variation of CLASI-D scores sorted by specific and non-specific cutaneous subtypes.

CLASI-D remained stable at 36 months compared to baseline for all specific and non-specific skin phenotypes.

On one hand, in the overall analysis of all cutaneous subtypes, there were no significant differences in CLASI-D values at various time points (p-value = 0.089).

On the other hand, considering the specific and non-specific cutaneous subtypes individually, the analysis of variance reported non-significant p-values at various time points. Table XXIII shows the variation at 36 months compared to baseline. To be more exhaustive, specific subtypes showed stability in CLASI-D scores at 36 months compared to baseline. Acute, subacute, and chronic lupus showed the following p-values, respectively: p = 0.508, p = 1.000, p = 0.770.

5. Cutaneous remission on specific subtypes

		Acute			Subacute			Chronic			
Total A.S.C.	**Skin phenotype**	TOT	**n°**	%	TOT	**n°**	%	TOT	**n°**	%	p-value
	CLASI A=0										
53	CLASI A=0 at 6 months	72	**34**	47,2	46	**16**	34,8	12	**3**	25,0	0,206
69	CLASI A=0 at 12 months	70	**42**	60,0	46	**23**	50,0	11	**4**	36,4	0,261
66	CLASI A=0 at 18 months	54	**41**	75,9	39	**22**	56,4	9	**3**	33,3	0,018
62	CLASI A=0 at 24 months	48	**39**	81,3	37	**19**	51,4	9	**4**	44,4	0,006
54	CLASI A=0 at 30 months	42	**31**	73,8	30	**19**	63,3	8	**4**	50,0	0,347
50	CLASI A=0 at 36 months	34	**30**	88,2	27	**17**	63,0	8	**3**	40,0	0,015

Table XXIV: CLASI-A remission stratified for different skin phenotypes. P values were assessed by Chi-squared test with Bonferroni correction.

Cutaneous remission in non-specific manifestations hasn't been evaluated because the results on efficacy weren't significant in this group.

6. GCs sparing effect induced by belimumab in cutaneous subtypes

6.1. GCs sparing effect induced in the Italian cohort

6.1.1. Cutaneous overall

Daily PDN intake	Mean±SD	N. of patients
PDN mg/die	10,2±8,6	242
PDN_6 (mg/die)	6,5±5,7	225
PDN_12	5,2±5,4	200
PDN_18	4,6±5,2	171
PDN_24	3,9±3,6	154
PDN_30 mg/die	4,0±3,2	131
PDN_36 (mg/die)	4,2±4,7	108

Table XXV: daily prednisone intake: mean and SD= standard variation; number of patients with cutaneous manifestation considered, regardless of subtypes.

The reduction of daily prednisone intake was previously demonstrated in the real-life study BeRLiSS-JS (157). Results on table XXV confirm this evidence with p-value < 0,001.

6.1.2. Acute cutaneous lupus

Daily PDN intake	Mean ± SD	N. of patients
PDN mg/die	13,5±11,1	25
PDN_6 (mg/die)	6,1±3,0	25
PDN_12	5,3±4,8	25
PDN_18	3,6±2,0	25
PDN_24	3,6±3,5	25
PDN_30 mg/die	3,2±2,7	25
PDN_36 (mg/die)	3,3±2,8	25

Table XXVI: daily prednisone intake described by mean and SD= standard variation; a valid number of patients with acute cutaneous lupus was considered.

The reduction of daily prednisone intake was significant in patients with acute cutaneous lupus (p-value < 0,001)

6.1.3. Subacute cutaneous lupus

Daily PDN intake	Mean±SD	N. of patients
PDN mg/die	9,7±5,4	19
PDN_6 (mg/die)	5,2±3,2	19
PDN_12	4,9±2,1	19
PDN_18	5,1±3,2	19
PDN_24	4,3±2,4	19
PDN_30 mg/die	4,7±2,5	19
PDN_36 (mg/die)	3,8±2,1	19

Table XXVII: daily prednisone intake described by mean and SD= standard variation; a valid number of patients with subacute cutaneous lupus was considered.

The reduction of daily prednisone intake was significant in patients with subacute cutaneous lupus (p-value < 0,001)

6.1.4. Chronic cutaneous lupus

Daily PDN intake	Mean±SD	N. of patients
PDN mg/die	11,3±1,7	2
PDN_6 (mg/die)	7,5±0,0	2
PDN_12	3,8±1,8	2
PDN_18	4,4±0,9	2
PDN_24	5,0±3,5	2
PDN_30 mg/die	5,0± 0,0	2
PDN_36 (mg/die)	3,1±0,9	2

Table XXVIII: daily prednisone intake described by mean and SD= standard variation; a valid number of patients with chronic cutaneous lupus was considered.

The reduction of daily prednisone intake was not significant in patients with chronic cutaneous lupus (p-value =1,000).

6.1.5. Cutaneous vasculitis

Daily PDN intake	Mean ±SD	N. of patients
PDN mg/die	13,8±7,8	4
PDN_6 (mg/die)	6,9±6,3	4
PDN_12	11,9±9,0	4
PDN_18	8,8±7,5	4
PDN_24	6,9±3,8	4
PDN_30 mg/die	8,1±4,7	4
PDN_36 (mg/die)	4,7±0,6	4

Table XXIX: daily prednisone intake described by mean and SD= standard variation; a valid number of patients with cutaneous vasculitis was considered.

The reduction of daily prednisone intake was not significant in patients with isolated cutaneous vasculitis (p-value =1,000)

6.1.6. Livedo reticularis

Livedo reticularis reported only one patient valid for the analysis, so no data were generated.

6.1.7. Alopecia/lupus hair

Daily PDN intake	Mean ±SD	N. of patients
PDN mg/die	10,0±0,0	2
PDN_6 (mg/die)	5,0±3,5	2
PDN_12	4,1±3,0	2
PDN_18	3,1±2,6	2
PDN_24	3,1±2,6	2
PDN_30 mg/die	3,8±1,8	2
PDN_36 (mg/die)	2,5±3,5	2

Table XXX: daily prednisone intake described by mean and SD= standard variation; a valid number of patients with alopecia/lupus hair was considered.

The reduction of daily prednisone intake was not significant in patients with isolated alopecia/lupus hair (p-value =1,000)

6.2. GCs sparing effect induced in the Padua cohort

In the Padua cohort, 126 patients were investigated for the amount of daily prednisone intake at the beginning of belimumab treatment and every six months.

6.2.1. GCs sparing effect in acute cutaneous subtype

Considering only patients with cutaneous manifestations and dividing them by subtype, we observed at baseline that, for patients with acute manifestations, the percentage of patients with 0 mg/day and 0.1-5 mg/day prednisone intake were 8.33% and 36.11% respectively. Throughout the follow-up there was a decrease in the dose of daily prednisone. At 36 months, 54.55% and 45.45% of patients were taking 0 and 0.1-5 mg/day of prednisone, respectively.

	T0	**T6**	**T12**	**T18**	**T24**	**T30**	**T36**
Number of patients							
0 mg/day	3	4	8	8	9	9	6
0,1-5 mg/day	13	16	15	9	6	4	5
5,1-7,5 mg/day	2	6	4	2	0	2	0
>7,5 mg/day	18	4	0	0	1	0	0
Total	36	30	27	19	16	15	11
Rates							
0 mg/day	8,33%	13,33%	29,63%	42,11%	56,25%	60,00%	54,55%
0,1-5 mg/day	36,11%	53,33%	55,56%	47,37%	37,50%	26,67%	45,45%
5,1-7,5 mg/day	5,56%	20,00%	14,81%	10,53%	0,00%	13,33%	0,00%
>7,5 mg/day	50,00%	13,33%	0,00%	0,00%	6,25%	0,00%	0,00%
Total	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**

Table XXXI: Acute patients were divided into prednisone intake ranges every 6 months during the follow-up period. Relative percentages have been also reported.

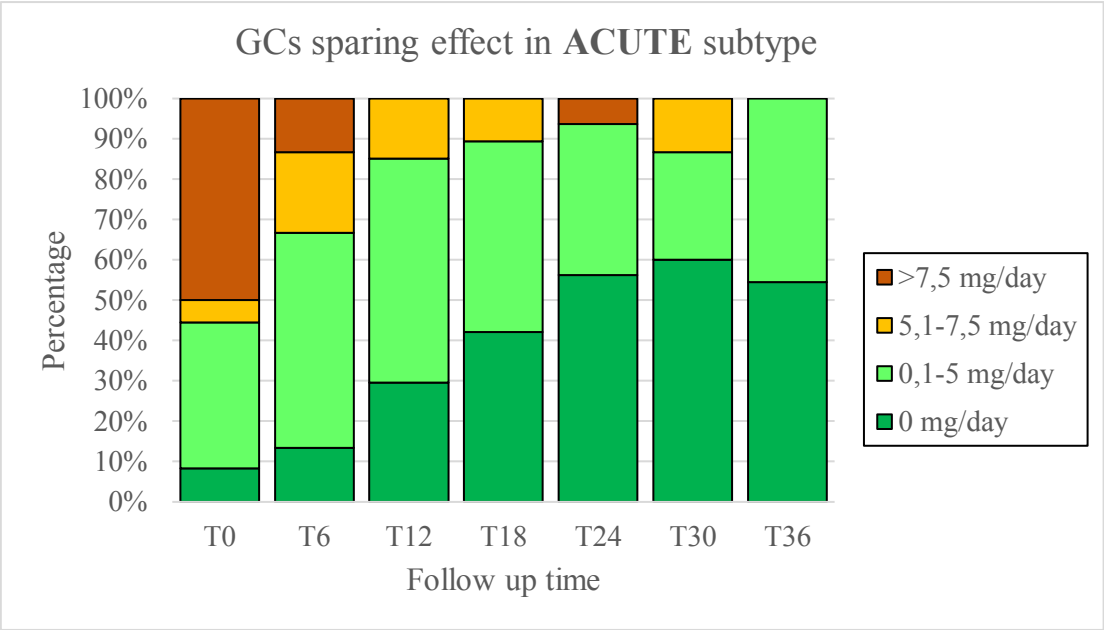


Figure 19: GCs sparing effect induced by belimumab in Padua patient's cohort on acute skin manifestations.

6.3. GCs sparing effect in subacute cutaneous subtype

Similarly, in patients with subacute manifestations, if at the beginning the percentage of patients with 0 mg/day and 0.1-5 mg/day prednisone intake was 4.17% and 25.00% respectively, then there was a mean decrease in daily prednisone intake throughout the follow-up. At 36 months, 9.09% and 81.82% of patients were taking 0 mg/day and 0.1-5 mg/day of prednisone, respectively.

	T0	**T6**	**T12**	**T18**	**T24**	**T30**	**T36**
Number of patients							
0 mg/day	1	1	1	2	0	1	1
0,1-5 mg/day	6	9	12	13	12	10	9
5,1-7,5 mg/day	4	7	8	2	2	2	1
>7,5 mg/day	13	6	0	1	0	0	0
Total	24	23	21	18	14	13	11
Rates							
0 mg/day	4,17%	4,35%	4,76%	11,11%	0,00%	7,69%	9,09%

0,1-5 mg/day	25,00%	39,13%	57,14%	72,22%	85,71%	76,92%	81,82%
5,1-7,5 mg/day	16,67%	30,43%	38,10%	11,11%	14,29%	15,38%	9,09%
>7,5 mg/day	54,17%	26,09%	0,00%	5,56%	0,00%	0,00%	0,00%
Total	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**

Table XXXII: Subacute patients were divided into prednisone intake ranges every 6 months during the follow-up period. Relative percentages have been also reported.

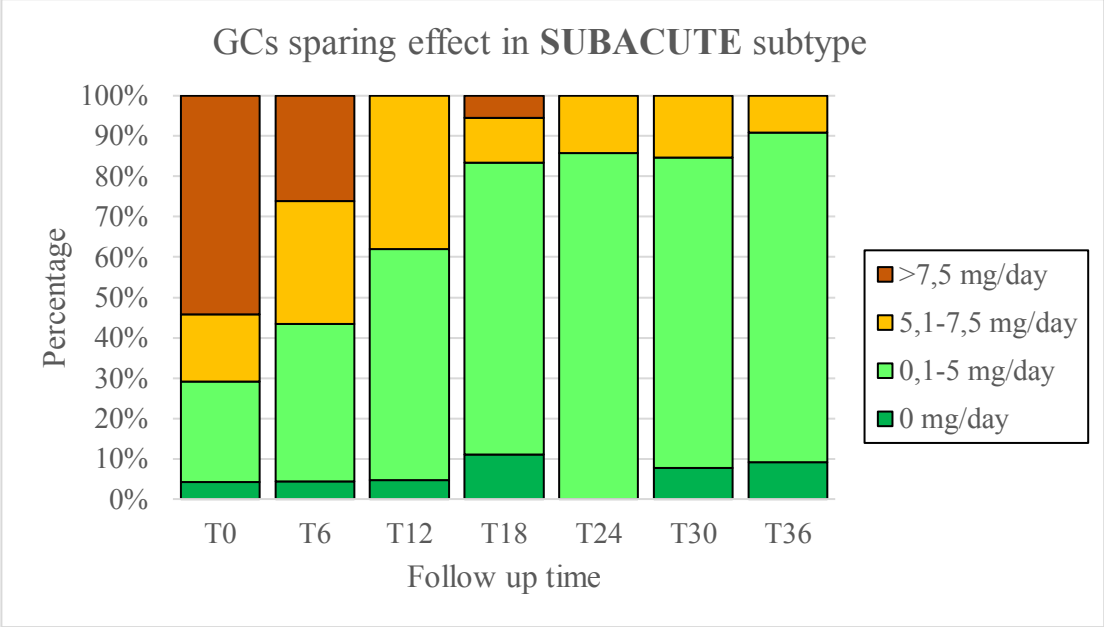


Figure 20: GCs sparing effect induced by belimumab in Padua patient's cohort on subacute skin manifestations.

6.4. GCs sparing effect in chronic cutaneous subtype

In patients with chronic skin manifestations, at the beginning the rate of patients with 0 mg/day and 0.1-5 mg/day of prednisone was 4.17% and 25.00% respectively. During the follow-up, there was a fluctuating trend in the doses of prednisone administered. However, despite no observed increase in the percentage of patients taking 0 prednisone, there was a relative increase in the number of patients taking 0.1-5 mg of prednisone per day.

	T0	T6	T12	T18	T24	T30	T36
Number of patients							
0 mg/day	1	0	0	0	0	0	0
0,1-5 mg/day	2	3	2	2	1	2	1
5,1-7,5mg/day	1	2	0	0	2	0	0
>7,5 mg/day	2	0	1	1	0	1	1
Total	6	5	3	3	3	3	2
Rates							
0 mg/day	16,67%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
0,1-5 mg/day	33,33%	60,00%	66,67%	66,67%	33,33%	66,67%	50,00%
5,1-7,5mg/day	16,67%	40,00%	0,00%	0,00%	66,67%	0,00%	0,00%
>7,5 mg/day	33,33%	0,00%	33,33%	33,33%	0,00%	33,33%	50,00%
Total	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**

Table XXXIII: Patients were divided into prednisone intake ranges every 6 months during the follow-up period. Relative percentages have been also reported.

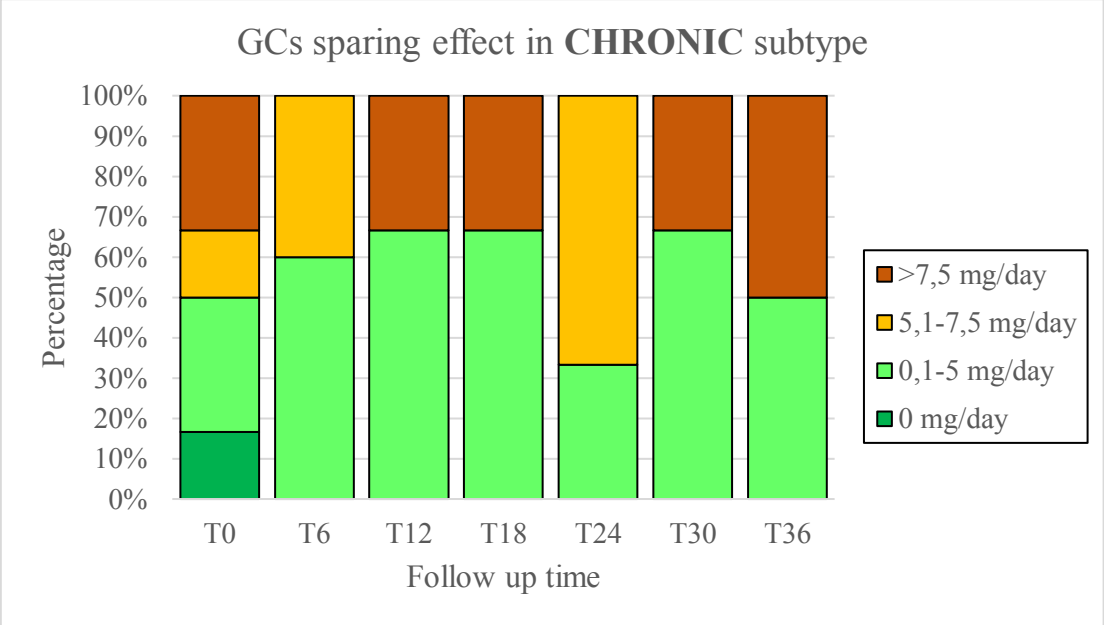


Figure 21: GCs sparing effect induced by belimumab in Padua patient's cohort on chronic skin manifestations.

DISCUSSION

The BeRLiSS-Skin study has confirmed in clinical practice the high efficacy of belimumab in patients with cutaneous involvement, further corroborating efficacy data from randomized controlled trials and previous real-life studies (e.g., BeRLiSS, BeRLiSS-JS(155–157)).

Belimumab inhibits the survival of B cells, also acting on autoreactive B cells, and has the capacity to reduce the differentiation of B cells into Ig-producing plasma cells. This provides a rationale for its use in SLE, supported by evidence of systemic and local overexpression of BLyS in SLE. Local overexpression of BLyS and BAFF-R has been demonstrated in various tissues, including keratinocytes from the skin lesions of SLE patients. Therefore, belimumab inhibits BLyS overexpression in the tissues of SLE patients.

The BeRLiSS-Skin study aimed to investigate whether this efficacy varied among different subtypes of cutaneous SLE.

In our multicentric cohort, we observed a difference in response in terms of efficacy measured with CLASI-A. Among patients with specific cutaneous manifestations, the group of patients with ACLE and SCLE showed differences compared to the group with chronic manifestations. This is because, in ACLE and SCLE, the CLASI-A decrease reached statistical significance ($p<0.001$) as early as 6 months, whereas in CCLE, a statistically significant decrease in CLASI-A was achieved as late as 12 months after starting belimumab treatment.

This suggests that patients with chronic lupus exhibit differences in terms of response and maybe in disease mechanisms.

Moreover, for nonspecific manifestations, the CLASI-A decrease was observed as late as 18 months for livedo reticularis (0-12 months $p=0.066$, 0-18 months $p=0.027$). However, no significant decrease in CLASI-A was found for the other nonspecific skin manifestations of SLE. However, for future prospects, expansion of cohort of these patients could lead to results with greater statistical strength.

This suggests, as the BerLiSS and BeRLiSS-JS studies showed (with SRI-4), that 6 months might not be sufficient to fully evaluate belimumab's efficacy (154,155).

According to our BeRLiSS-Skin data, a specific evaluation of efficacy could already be made at 6 months for acute and subacute SLE, while for chronic SLE could be appropriate to wait for 12 months.

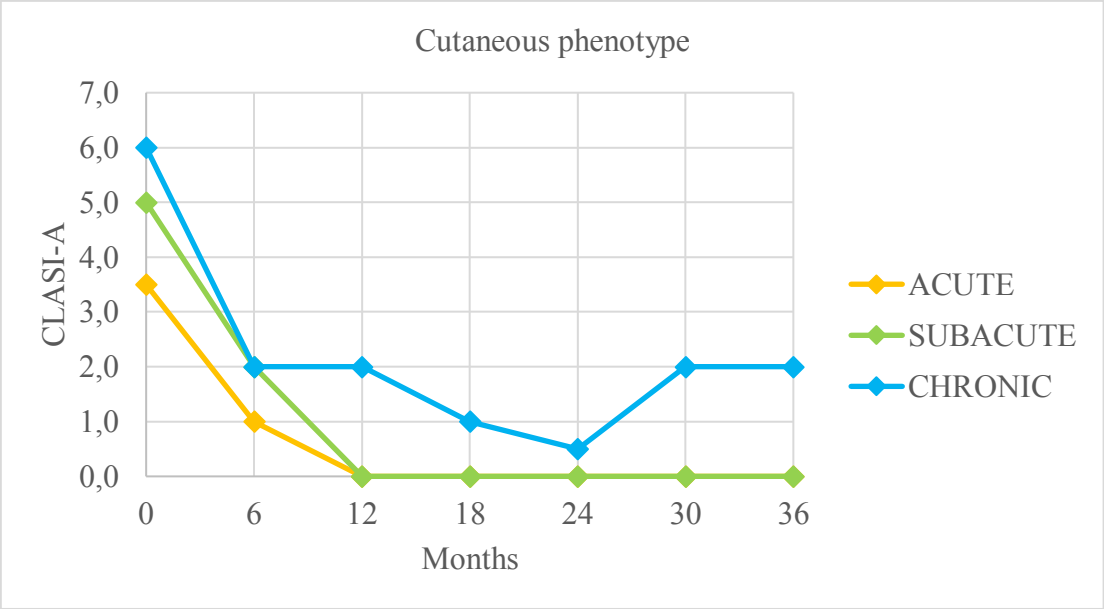


Figure 22: Decrease of median CLASI-A values stratified by skin phenotypes.

It's interesting to note that the decrease of median values are more rapid and stable in acute and subacute subtypes, whereas in chronic lupus the response tend to be delayed compared to the other two subgroups and with the possibility of flares during the follow-up.

Regarding the nonspecific cutaneous sample size, it was sufficient (48 patients for cutaneous vasculitis, 23 patients with livedo, 79 patients for alopecia/lupus hair), but few of these were purely nonspecific. By including all of them, we would have risked to consider patients who might have been influenced by the specific SLE subtype rather than by the nonspecific one, introducing confounding factors into the p-value of improvement.
Consequently, few patients meet the inclusion criteria to be purely non-specific and, as a result, the analyses have limited strength in this context.

Additionally, from the results evaluating remission in SLE patients with cutaneous manifestations, disparities were observed among the three specific subtypes. The study showed that ACLE achieves remission (CLASI-A=0) more frequently in percentage if compared to SCLE and CCLE. This indicates patients with acute lupus as potentially better responder to belimumab in terms of both CLASI-A decrease and speed of achieving clinical remission. The trend of remission increases in time for all three specific cutaneous subtypes, but the timing of remission is different: 47.2% of ACLE patients achieved remission in 6 months of treatment. In SCLE, 50% of patients achieved remission only after 12 months, while 50% of CCLE patients reached remission at 30 months from the start of belimumab.

It can be concluded that remission induced by belimumab can be achieved by all three subtypes but exhibit a difference in remission velocity, which is higher in the acute subtype.

Regarding nonspecific manifestations, remission significance as p-value was not studied because, in turn, CLASI-A decrease was not significant.

According to the BeRLiSS-JS study in patients with skin involvement, the lack of CLASI-A early remission during belimumab treatment did not prevent its achievement later during the follow-up (157). This finding was confirmed in our study, where patients achieved remission through an increasing trend in all three subgroups, as explained in figure 24.

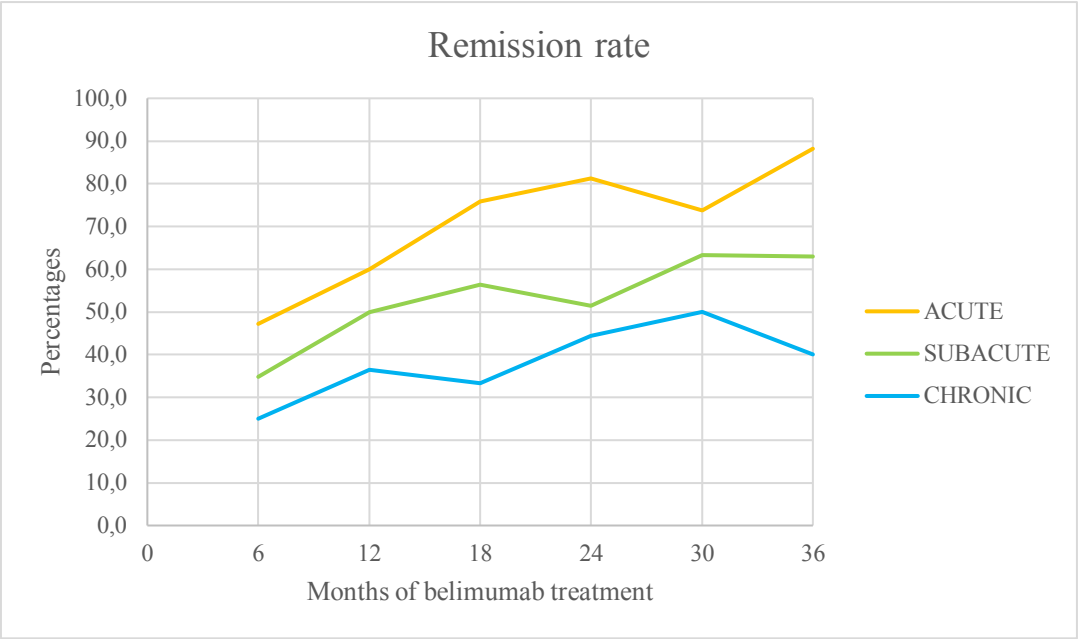


Figure 23: remission rate sorted by specific skin subtypes manifestations.

According to our results, CLASI-A remission was more frequent in patients with acute than subacute and chronic phenotypes with significance at 18, 24, and 36 months. In other words, having acute cutaneous lupus implies a better response to belimumab in terms of disease remission if compared to the respective remission rate achieved by patients with subacute and chronic subtypes.

Moreover, if in BeRLiSS (155) damage accrual with belimumab treatment didn't increased significantly in patients with a baseline SDI score of 0 at 12, 24, and 36 months, in our study, regarding skin manifestations, CLASI-D scores (expressing skin damage) remained stable at 36 months compared to baseline for all specific and non-specific skin phenotypes (p-value = 0.089).

A glucocorticoid-sparing effect of belimumab on overall skin manifestation was also shown (p<0,001). There was a decrease in the daily GCs dose and a consistent proportion of patients experiencing prednisone withdrawal during the follow-up, thereby lowering the cumulative glucocorticoids intake. BeRLiSS-Skin confirmed the glucocorticoid-sparing effect of belimumab as stated in BeRLiSS-JS(157).

A more interesting result was achieved by considering patients with cutaneous manifestations and dividing them by subtype.

GCs sparing effect of belimumab was significant for patients with acute ($p<0,001$) and subacute ($p<0,001$) lupus, whereas for the chronic subtype, cutaneous vasculitis, livedo reticularis, and alopecia/lupus hair subtypes, the variation in daily average PDN intake did not yield significant results.

Furthermore, in patients with acute skin manifestations after 3 years of therapy, 93.5% of patients remained in treatment, had prednisone levels compliant with the EULAR recommendations (132). In patients with subacute disease if compared to acute cases, a higher percentage of patients had to maintain GCs rather than undergo withdrawal, which could suggest a persistence of disease activity requiring prednisone. Furthermore, this trend was even more pronounced when comparing chronic subtype with the acute one.

Notably, the decrease in the cumulative intake of glucocorticoids is one of the main goals in the modern management of SLE (132) in order to prevent long-term damage accrual and to improve quality of life. (157).

In conclusion, we can define acute cutaneous lupus as the subtype that shows the best response in terms of efficacy, clinical remission, and reduction in daily prednisone intake when compared to other cutaneous subtypes of lupus.

The study has both strengths and limitations. Limitations include the lack of a control group, the exclusion of patients for whom data weren’t available at any time, the cohort's variability over time due to therapy discontinuation and the absence of patient-reported outcomes (PROs). These limitations are mainly related to the retrospective nature of the study, which poses some objective restrictions on the amount of data that can be inferred. Another limitation is the small number of both patients with nonspecific manifestations and with CCLE. The small sample size of some statistical groups is due to epidemiology of SLE. This is relevant especially for CCLE because their majority exhibit only cutaneous manifestations, and only 5-10% of cases present systemic symptoms. Therefore, systemic therapy with belimumab is rarely adopted in CCLE since in most cases only topical therapy is sufficient.

The main strengths of the study are its real-world context, the large cohort of patients analysed in accredited centres following EULAR guidelines and the long duration of follow-up. Furthermore, statistical analyses avoided selection bias in remission's analysis maintaining as denominator the previous 6 months and not only those considered still on treatment. In this way we also considered those who, for inefficacy or safety reasons discontinued belimumab.

CONCLUSIONS

In this study we demonstrate that belimumab was effective at reducing active skin manifestations in all specific skin lesions (acute, subacute, chronic). On one hand, CLASI-A reduction was achieved at 6 months for the acute and subacute phenotype, and later at 12 months in the chronic phenotype. CLASI-D stability hints at a lessened damage accrual over the span of 36 months across all skin specific phenotypes. On the other hand, patients with acute skin subtype achieve more frequently CLASI-A remission and glucocorticoid withdrawal than those with other phenotypes; therefore, we can assume ACLE as the best responder profile to belimumab in terms of time-efficacy, time to remission and glucocorticoid withdrawal.

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